IDENTIFICATION AND CHARACTERIZATION OF THERMOTOLERANCE IN LETTUCE SEED GERMINATION

By

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IDENTIFICATION AND CHARACTERIZATION OF THERMOTOLERANCE DURING
LETTUCE SEED GERMINATION

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Botanically, the seed of lettuce is an achene. For germination to occur, the embryonic axis must penetrate the endosperm, integument, and pericarp. At high temperature, germination of lettuce seed is inhibited. The objective of this research was to investigate how lettuce seeds bypass thermoinhibition in order to germinate at supraoptimal temperatures.

Germination tests were conducted on the thermogradient bar in order to identify the role of temperature during seed development on the subsequent alteration of the level of thermotolerance during germination. 'Dark Green Boston' and 'Valmaine' were found to be thermosensitive and 'Floricos 83', 'Everglades', and PI 251245 thermotolerant. Seeds of the five genotypes were matured at four growth temperatures. Seeds matured at 30°/20°C or 35°/25°C had greatly enhanced seed germination at 36°C compared to those matured at 20°/10°C or 25°/15°C. Seed priming also overcame thermoinhibition of both thermosensitive and thermotolerant genotypes.

A puncture test was used to determine the role of the seed coverings in restricting seed germination at high temperature. Primed seeds or seeds of thermotolerant genotypes required less force to penetrate the seed coverings, especially the endosperm, at high temperature as germination proceeded toward radicle protrusion.

For seeds that overcame thermoinhibition, the structural alterations of endosperm in the micropylar area during radicle emergence at 36°C were observed. The endosperm layer separated from the integument, protein bodies depleted, empty vacuoles formed, and the cytoplasm condensed; the endosperm cell walls ruptured and the embryo grew toward this opening. The papery endosperm layer was weakened by priming. Seeds matured at 30°/20°C enhanced disruption and rupture of the endosperm cell wall compared to seeds matured at 20°/10°C.

Thermotolerance of seed was increased by manipulating temperature during seed production. The endosperm was identified as the physical barrier on seed germination at supraoptimal temperatures. Genotype, seed maturation temperature, and seed priming could release thermoinhibition on germination. In all cases, a reduction of physical resistance by endosperm cell wall weakening and dissolution of stored reserves in endosperm cells led to germination at 36°C. Weakening the endosperm layer was a prerequisite to radicle protrusion at high temperature and became the central theme to thermosensitivity.

CHAPTER 1
INTRODUCTION

Lettuce (*Lactuca sativa* L.) is an important salad crop produced year-round in the United States. The seed of lettuce is actually a fruit (achene). The tissues that surround the embryo of the achene include the pericarp, integument, and endosperm. The primary method of planting lettuce is by direct seeding. When lettuce seeds are sown in late summer or early fall, the soil temperature is often above 30°C, which is above the optimum temperature range (15° to 22°C) for lettuce seed germination, and often exceeds the maximum range for many genotypes (Gray, 1975). As a result, germination of lettuce seeds can be erratic or completely inhibited and crop production is therefore limited.

When lettuce emergence in the field is slow and irregular, ununiform stands may result and, if a once-over harvest operation is employed, yield is reduced. If multiple harvests occur, such uneven stands may present problems in the management of cultivation. In addition, poor stands allow growth of weeds which will compete for resources needed for lettuce growing. One approach to addressing these problems is to increase the ability of seed to germinate under conditions of high soil temperatures. By increasing thermotolerance in seed, one can improve both the uniformity of stand establishment in the field and the subsequent yield.

Lettuce seed germination is strongly temperature dependent. As temperature rises even 2 or 3°C above the optimum, germination can sharply decline from nearly 100% to near 0% (Reynolds and Thompson, 1971), a phenomenon known as thermoinhibition. This inhibition of germination upon imbibition at a supraoptimal temperature is not permanent. If the temperature returns to an appropriate level for

germination, the seeds are able to resume germination. An increased period of imbibition during supraoptimal temperatures may induce a secondary dormancy called thermodormancy (Khan, 1980/81). In this case, the seeds become dormant and will not germinate even if they are returned to favorable temperatures for germination. Thermoinhibition is a more transient condition than thermodormancy, and releasing the thermoinhibition in lettuce seed can prevent the induction of thermodormancy.

The problems of thermoinhibition and thermodormancy in lettuce seed have been the subject of interest to researchers. At high temperature, inhibition of seed germination of lettuce has been attributed to several factors. These factors include: a) reduction of seed covering permeability to oxygen and carbon dioxide (Borthwick and Robbins, 1928) b) physical barriers within the seed to water uptake (Speer, 1974) c) accumulation of metabolic products in the endosperm or embryo (Borthwick and Robbins, 1928) d) inhibitory effects of abscisic acid (McWha, 1976) e) mechanical restraint of the seed coverings (Ikuma and Thimann, 1963b) f) inhibition of the secretion of cell-wall-weakening enzymes (Ikuma and Thimann, 1963b) g) deficiency of the growth potential of the embryo (Nabors and Lang, 1971a) and h) nonfunction of phytochrome (Scheibe and Lang, 1969).

Emerging radicles of lettuce can develop enough thrust to overcome the estimated mechanical resistance of the endosperm without first degrading the endosperm cell wall (Nabors and Lang, 1971a, b). Also, cell-wall-degrading enzymes located at the micropylar region of the endosperm tissue may be activated and may be a necessary prerequisite for normal germination (Ikuma and Thimann, 1963b). During germination, weakening of endosperm tissue around the radicle just prior to radicle protrusion has been identified in pepper (Watkins and Cantliffe, 1983) and tomato (Groot and Karssen, 1987) and may apply to lettuce. The major components of the cell wall of endosperm tissue in the micropylar region of lettuce seed are mannose, arabinose, and glucose (Dutta et al., 1994). Mannanase, cellulase, pectinase, and

pentosanase can be effective in the promotion of germination of dormant lettuce seed, although it has not been established that these enzymes mediate endosperm wall weakening *in vivo* prior to radicle emergence (Bewley and Halmer, 1980/81; Halmer et al., 1976; Ikuma and Thimann, 1963b).

During seed production, both lettuce genotype and environmental conditions may affect the inhibition of seed germination at high temperature. The critical maximum temperature for germination in lettuce seeds depends on genotype (Borthwick and Robbins, 1928). In this sense, most crisphead lettuce types germinate more successfully than butterhead types at temperatures above 28 to 30°C (Gray, 1975). A wild plant accession, PI 251245, has been identified to be a thermotolerant line (Bradford, 1985). During lettuce seed maturation, ambient environmental temperature may affect subsequent germination of seeds (Damania, 1986; Drew and Brocklehurst, 1990; Gray et al., 1988; Harrington and Thompson, 1952; Koller, 1962). In this regard, seeds collected from hot climatic zones tend to germinate at higher temperatures, suggesting that manipulating the environment during seed development and maturation can potentially increase thermotolerance in lettuce seeds (Damania, 1986; Harrington and Thompson, 1952).

Seed priming, a controlled hydration process that permits pregerminative metabolic activity to proceed but prevents radicle emergence (Heydecker et al., 1975), is one of the most effective methods to overcome thermodormancy in seed of a number of crops (Atherton and Farooque, 1983; Gelmond, 1965; Nakamure et al., 1982). Seed priming can successfully overcome thermoinhibition/thermodormancy in several varieties of lettuce seeds (Cantliffe, 1981; Cantliffe et al., 1981; Guedes and Cantliffe, 1980). Because seed priming can shorten the time from sowing to seedling emergence, the process of seed germination can be completed before environmental fluctuations become injurious to seedling establishment (Khan, 1977).

To better understand the thermoinhibition process, further studies are required to identify the mechanisms controlling thermotolerance in lettuce seeds. Therefore, it would be useful to evaluate lettuce germplasm to identify various genotypes that may be thermotolerant. Understanding how such genotypes may be more thermotolerant could be correlated to such processes as seed priming to overcome thermoinhibition/thermodomancy.

The objective of this research was to investigate how lettuce seeds bypass thermoinhibition/thermodormancy in order to germinate at supraoptimal temperatures. Three approaches were used in the investigation. These were: 1) identifying genotypes that have different germination temperature maximums, including thermotolerance; 2) studying those genotypes as they develop and mature their seed under various temperature environments; and 3) refining the seed priming technique. These approaches could then be correlated to such changes as ultrastructure of the seeds during imbibition at high temperature and fruit coat-endosperm resistance to germination.

CHAPTER 2
REVIEW OF LITERATURE

The purpose of the following review is to discuss the metabolics of lettuce seed germination, the restriction of lettuce seed germination at high temperature, and the methods of relieving thermodormancy. This review is an overview of the physiological and biochemical mechanisms of thermotolerance in lettuce seed.

The Lettuce Seed and Germination

Germination of lettuce seed (achene) has been studied for many decades. Light and temperature are two major environmental factors affecting germination of lettuce seed. Under unfavorable environmental conditions, seed dormancy may be induced, for example, if the seeds are imbibed in darkness or at high temperature (Ikuma and Thimann, 1964; Toole et al., 1956;). Relief of dormancy can be obtained by a number of methods, such as exposure to red light (Scheibe and Lang, 1964), application of plant growth regulators or chemicals (Dunlap and Morgan, 1977; Keys et al., 1975; Sanini et al., 1986), and seed priming (Cantliffe et al., 1981). The physiology of the germination of lettuce seed is complex. Researchers have described the mechanical, physiological, and biochemical mechanisms in the germination of seed.

Structure

Borthwick and Robbins (1928) described the structure of the seed coverings that surround the embryo of the mature achene as follows:

1. The pericarp, also called the fruit coat, is the outer surface of the seed. It is a nonliving, ribbed structure consisting of thick-walled, lignified cells.

2. The integument, a term which refers to the seed coat, is composed of the remnants of the outer epidermis and some parenchymatous cells. It forms a nonliving layer between an inner, suberized, semipermeable membrane and an outer, thick-walled epidermis.

3. The endosperm, a living tissue (8% of seed dry weight), adheres to the inner epidermis of the integument. Most of the endosperm is two cells thick except at the radicle end, which is three or more cells thick. The cell wall of the endosperm is thick and has numerous column-like protuberances.

The major components of the cell wall are manno-polysaccharides (carbohydrates, 3.4% of seed dry weight). The cytoplasm of the endosperm cell contains abundant protein bodies (0.23% of seed dry weight) and lipid storage material (4.3% of seed dry weight) (Halmer et al., 1978; Leung et al., 1979). The endosperm acts as a restriction or barrier for embryo growth and provides nutrients for growing seedlings following germination (Abeles, 1986).

The cotyledons are another major storage tissue (60-70% of seed dry weight). Most stored reserves in cotyledons are lipid (27% of seed dry weight) and protein (2.7% of seed dry weight), with small amount of phytate, soluble sugars, and very little starch. The reserves in cotyledons do not mobilize until stored reserves in the endosperm have been degraded (Halmer et al., 1978; Leung et al., 1979).

Germination

Germination is defined as the emergence of the radicle through the surrounding seed coverings. The major events that occur in germination of lettuce seed include water imbibition, enzyme activation, storage tissue breakdown, initiation of embryo growth, rupture of the seed coverings, and establishment of the seedlings (Copeland

and McDonald, 1985). The processes of germination occur close to the tip of the embryonic axis (Ikuma and Thimann, 1964).

As lettuce seeds imbibe in water, some of the most significant changes in the embryo occur in the plastids (Srivastava and Paulson, 1968). After a short period of soaking, well-defined and fairly large plastids appear in all tissues. In the hypocotyl, plastids reveal a great deal of change but do not undergo much change in size or appearance at the root tip. Reserve proteins and lipids fill the dry embryo cells. The protein and lipid bodies are gradually depleted and eventually disappear after imbibition

The order of events in the mobilization of stored reserves in the cotyledons, axis, and endosperm of germinated lettuce seeds has been established. The endosperm is the initial source of food reserves (proteins, lipids, and carbohydrates) for the growing embryo. Stored reserves (lipids, proteins, and phytate) in the cotyledons and axis mobilize after degradation of the endosperm (Leung et al., 1979). The degradation of endosperm requires a number of enzymes, which can be present as preformed enzymes or be synthesized *de novo* within the endosperm cells. The endosperm cells contain all of the cytological prerequisites for enzyme synthesis. For example, the protein bodies can store enzymes or proteins that provide amino acids or small peptides for the synthesis of new enzymes. Lipid-containing spherosomes can connect to the endoplasmic reticulum and sometimes bear ribosomes. In addition, free ribosomes and mitochondria can be found in the ground cytoplasm (Jones, 1974).

Under favorable germination circumstances, radicle emergence and seedling establishment are a result of cell expansion and cell division of the growing embryo (Toole et al., 1956). The expansion of radicle cells precedes mitosis by many hours during lettuce seed germination. It is cell expansion that results in radicle protrusion; cell division plays little or no role in the germination process (Haber and Luippold, 1960a). Srivastava and Paulson (1968) showed that, after 12 hours of imbibition, the first detectable cellular changes during the germination of 'Grand Rapids' occurred in

the epidermal and cortical cells about 0.5mm behind the root apex. These cells were activated and expanded, then the radicle bent and penetrated the seed coverings.

Environmental Factors

Light

Phytochrome is a photoreversible, photomorphogenetic pigment that exists in two forms: Pr, the red light-absorbing form, and Pfr, the far-red light-absorbing form. The Pfr form of phytochrome in hydrated tissue is unstable and can either undergo dark reversion to Pr or destruction, a process that represents a loss of photoreversibility (Butler et al., 1963; Toole et al., 1956).

The transformation between Pr and Pfr is affected by temperature. Low temperature prevents the transformation of a physiologically active Pfr form to an inactive Pr form (Scheibe and Lang, 1965). Evenari et al. (1953) have pointed out that there are possibly two limit points to the two operation mechanisms. One mechanism ensures that the germination of seed is not affected by light and operates up to the temperature limit below which germination can not be decreased by light alone. The other mechanism is light-sensitive, responding to the effects of red as well as far-red light and operates up to the temperature limit above which germination can not be increased by light alone. While photosensitive seeds sown over a suitable temperature range can germinate in darkness, at supraoptimal temperature a short exposure to red light after a period of imbibition is required to induce germination.

Fielding et al. (1992) showed that there was a close link between phytochrome action and the upper temperature limit for seed germination. Increasing Pfr levels in seed caused a successive increase in the upper temperature limit for germination. Kristie and Fielding (1994) indicated that the Pfr level required to induce 50% germination of lettuce seeds after a single light plus treatment increased from about

11% at 15° and 20°C to 86% at 30.5°C. Following a single light plus treatment, seed germination at high temperature was limited solely by the availability of Pfr. The precise site of upper temperature limit for seed germination was influenced by reversion of the Pfr form to the Pr form. The block to germination at temperatures above the upper limit resided within the steps governing escape from photoreversibility rather than the later steps in the transduction chain between Pr and Pfr.

Germination of seeds of some lettuce genotypes, such as 'Grand Rapids', is controlled by light. Photosensitive lettuce seeds having a functional phytochrome system are affected in their germination responses following red or far-red light irradiation (Borthwick et al., 1952). When 'Grand Rapids' seeds were imbibed at 18°C in darkness, there was a maximum germination which could not be further increased by red light. At about 32°C, the seeds would not germinate in darkness and this could not be improved by red light. With these temperature limits, the maximum effect of red light on germination was reached at approximately 26°C (Evenari et al., 1953).

Red light increases lettuce seed germination as seed hydration increases from 8 to 15%; it reaches a maximum at moisture contents above 18%. Seeds have no response to red light at moisture contents below 8% (Hsiao and Vidaver, 1971; Vertucci et al., 1987). Seeds that have been imbibed and irradiated with red light can be redried without affecting subsequent viability. The redried seeds will germinate in darkness and can be inhibited by a short exposure to far-red light after imbibition (Vidaver and Hsiao, 1972). This suggests that phytochrome in mature lettuce seed is primarily in the Pr form, and photoconversion to Pfr can not happen in the dehydrated state (Kendrick and Russell, 1975).

The photoreceptive site in phytochrome-mediated lettuce seed germination corresponds to the tip of the hypocotyl (Ikuma and Thimann, 1959). This site is at the upper half of the embryonic axis and never forms root hairs. At the beginning of the imbibition period, red light-induced growth occurred simultaneously in the hypocotyl and

the radicle. Therefore, the light signal is probably transferred from the hypocotyl to the root (Inoue and Nagashima, 1991).

Germination of light-sensitive lettuce seeds can be stimulated or inhibited by applying plant growth regulators (Ikuma and Thimann 1963a; Kahn, 1960; Khan, 1968). Irradiation causes the production of gibberellins (GAs) in lettuce seed. The GAs are involved in phytochrome-mediated growth responses in the axis (Kahn et al., 1957). Both GAs and light induce changes in water potential of the embryonic axis of the seed, although gibberellin-promoted seed germination cannot be reversed by a period of far-red irradiation. The seed's response to GAs is probably achieved in a way different from its response to irradiation (Ikuma and Thimann, 1960; Kahn and Goss, 1957; Scheibe and Lang, 1965).

Abscisic acid (ABA) is a potent inhibitor of seed germination. ABA within both the dry, non-dormant and the dormant lettuce seed can inhibit germination. In non-dormant seed, the amount of ABA falls sharply during imbibition (McWha and Hillman, 1974). The inhibition of seed germination correlates with the amount of endogenous ABA or exogenously applied ABA (McWha, 1976). Endogeneous ABA may be reduced by leaching as well as by metabolic degradation (Dulson et al., 1988; McWha and Hillman, 1974).

Cytokinins can overcome inhibition of seed germination by ABA (Bewley and Fountain, 1972). They have minimal effect on germination in darkness, but promote germination in light (Miller, 1958). Khan and Tolbert (1965) reported that light was essential for cytokinin reversal of ABA inhibition in the light-requiring 'Grand Rapids' seeds but not in the light-insensitive 'Paris White' seeds. Germination of light-requiring seeds is modulated by GAs and requires the presence of cytokinins when seeds germinate under stressful conditions. Cytokinins act on cotyledon expansion, whereas GAs enhance axis elongation (Ikuma and Thimann, 1963a).

Temperature

The optimum temperature for lettuce seed germination varies with genotype. C, Although the optimum temperature for germination is between 15° and 22°C, most genotypes will germinate well at temperatures ranging from 5° to 25°. At temperatures above 25°C, germination rate decreases; above 27°C, both the rate and the percentage of germination are drastically reduced (Borthwick and Robbins, 1928). The upper temperature limit for most lettuce seed germination is between 28° and 32°C (Damania, 1986). When lettuce seeds are imbibed at high temperature, germination is inhibited, a phenomenon called thermoinhibition. These thermoinhibited seeds can still germinate, however, once they are returned to a lower imbibition temperature. When the seeds are kept at high temperature for an extended period of time, the induction of thermodormancy, a secondary dormancy, occurs (Khan, 1980/81). Lettuce seeds will not germinate once they are thermodormant, until that dormancy is overcome.

Gray (1977) reported that two stages of seed germination of 'Grand Rapids' lettuce were particularly sensitive to high temperature. The first stage occurred during the first four hours of imbibition; the second stage was between the beginning of mitosis and the onset of radicle emergence. The restrictions on germination by high temperature were different between the two phases. In the first phase, germination of seeds was strictly inhibited, but in the second phase, high temperature had relatively minimal effect on germination. Takeba and Matsubara (1976) indicated that seeds that were imbibed for a period of time at low temperature could germinate in high temperature conditions. Moreover, the longer the seeds were imbibed at 20°C, the higher their germination percentage was after they were transferred to high temperature. These results suggested that a thermo-labile factor controls the process of germination. When temperatures were above 30°C, the factor was inactivated, but it could be reformed or reactivated at 20°C.

Enzymatic Metabolism During Germination

Two important physiological and biochemical events take place in the imbibed lettuce seeds prior to radicle emergence. These are degradation of stored reserves in the endosperm and change in embryonic growth potential during germination.

Degradation of Stored Reserves in the Endosperm

Biochemical research on the lettuce endosperm during germination indicates that several changes occur about the time of radicle protrusion: appearance of enzymes and their functions initiated, mobilization of stored reserves and cell wall materials, and a decrease of endosperm weight (Psaras et al., 1981). Pavlista and Valdovinos (1978) used scanning electron microscopy to observe the disruptions on the endosperm of 'Grand Rapids' lettuce seed approximately four hours before germination. The cytoplasm of the micropylar endosperm cells underwent drastic changes even though the cell walls of the entire endosperm remained intact. The cytoplasm became highly vacuolated and reserve materials (proteins and lipids) were mobilized (Psaras et al., 1981).

Cell-wall-degrading enzymes located in the micropylar region of the endosperm tissue are activated during imbibition and this induction may be a necessary prerequisite for normal germination. This suggestion is supported by: a) the increase in germination caused by cutting the endosperm tissue (Ikuma and Thimann, 1963b), b) the embryo buckling within an unweakened endosperm (Pavlista and Haber, 1970), c) the endosperm disruptions at the micropylar region prior to the onset of germination (Pavlista and Valdovinos, 1978), d) increase in the activity of carboxymethylcellulase prior to endosperm degradation (Pavlista and Valdovinos, 1975), and e) the injection of cellulase and pectinase under the endosperm envelope, inducing significant germination (Ikuma and Thimann, 1963b).

The major stored reserves in the endosperm are the cell wall carbohydrates, particularly galactomannan (Halmer et al., 1975). After carrying out a series of research studies, Bewley and his coworker (Ouellette and Bewley, 1986) reported that mobilization of the endosperm cell wall reserves required several enzymes: endo-β-mannanase, α-galactosidase, and β-mannoside mannohydrolase. In cotyledons, β-mannoside mannohydrolase hydrolyzes mannobiose and mannotriose, which are the breakdown products of α-galactosidase and endo-β-mannanase action in the endosperm tissue. The action of α-galactosidase does not act upon the native endosperm cell wall but requires the action of β-mannanase, which is an important enzyme in hydrolyzing mannan.

Halmer et al. (1976) indicated that the amount of mannanase in germinated 'Grand Rapids' lettuce seeds was about 100 times greater than that in freshly imbibed ones. Germination of red-light or gibberellin-treated seeds began after 9-11 hours of imbibition, yet endo-β-mannanase activity did not increase until at least 4 hours later. When germination of phytochrome- and gibberellin-induced seeds was inhibited, however, the production of mannanase was minimal. Although mannanase obviously is the major degrading enzyme in the endosperm tissues, it is doubtful that it plays a role in weakening the cell wall of the endosperm since its amount rises after the radicle has emerged. Therefore, it is proposed that either other enzymes are involved in weakening the endosperm cell wall or some fine control mechanisms operate to assure the function of a low amount of mannanase that is present in both germinating and nongerminating seeds, but which is only active in germinating seeds (Bewley and Halmer, 1980/81).

Bradford and his coworkers analyzed the degradation products of the endosperm cell wall of hydrated lettuce seeds by an autolysis method (Dutta et al., 1994). They found that the composition of the cell-wall polysaccharides is significantly different in the micropylar and lateral endosperm regions and that the degradation products of cell walls in the micropylar region are rich in arabinose and glucose,

although those from the lateral region consist mostly of mannose. These differences suggest that wall-hydrolyzing enzymes other than mannanase may be involved in weakening micropylar endosperm in the preradicle emergence (Dutta et al., 1994). For example, Ikuma and Thimann (1963b) reported that cellulase, pectinase, and pentosanase were effective in the promotion of dormant lettuce seed germination.

These suggestions highlight the importance of two further studies: one to determine what critical enzyme is present before radicle emergence and the other to investigate whether there is any mechanism involved in weakening the cell wall of endosperm tissue.

Embryonic Growth Potential

Growth potential of the embryo is measured by the osmotic potential required to achieve 50% seed germination (Scheibe and Lang, 1965). A negative water potential in embryonic cells is essential for seed germination and is the principal driving force for cell expansion. Cells change osmotic potential (via solute production) or pressure potential (via increased wall loosening) to change their water potential (Ray et al., 1972). Solute accumulation prior to radicle growth can lower the osmotic potential and generate sufficient turgor pressure to allow the embryo to penetrate the endosperm barrier. The ability of the embryo to absorb water from its environment and to initiate growth is dependent on the osmotic potential of its cells (Nabors and Lang, 1971a,b; Takeba, 1980a). The time taken for a seed to initiate radicle growth is inversely proportional to the difference between the embryonic water potential during imbibition and its threshold water potential which prevents radicle growth: the greater the differences, the sooner the radicle growth will begin after imbibition (Bradford, 1990).

Germination of lettuce seed can be controlled by light, temperature, hormones, or a combination of these three. All can influence the growth potential in the embryonic

axes. Researchers have suggested that the phytochrome control of germination in positively photoblastic lettuce seed is mediated through increasing the growth potential of the embryo, measured by the osmotic potential required to achieve 50% seed germination (Nabors and Lang, 1971b; Scheibe and Lang, 1965). Nabors and Lang (1971b) measured the growth potential of the embryo using mannitol and polyethylene glycol as osmotica. Their results indicated that the force needed for the radicle to penetrate the seed coat was around an osmotic potential of 0.16 to 0.38 molar mannitol. In osmotica, light-treated embryos of positively photoblastic seeds developed a water potential 0.30 molar lower than that of dark-treated embryos; this was sufficient for seed germination. The phytochrome-mediated growth increase in the embryonic axes is an integrated function of the cells: increased wall loosening is coupled with rising osmotic constituents (Carpita et al., 1979a). Increasing the growth potential in the embryo can overcome the resistance offered by the endosperm (Nabors and Lang, 1971b).

Carpita et al. (1979b) indicated that the growth of embryonic axes and the degradation of stored reserves were not different in the red- and far red-treated seeds. However, axes of red-treated seeds increased their secretion of H^+ and their uptake of K^+ and Na^+ more than those of far red-treated seeds. A possible explanation for this increased secretion was that a phytochrome-stimulated proton pump initiated water-potential changes that allowed the embryos to relieve the mechanical restraint of the seed coverings.

Takeba (1980a) suggested that changes in the growth potential of the embryonic axes could be best explained by changes in the amount of an osmotic substance in the axes. The great accumulation of glutamate (Glu) and glutamine (Gln) in germinating seeds indicated that Glu and Gln possibly acted as osmotic substances in the germination of 'New York' lettuce seeds. Takeba (1980b) reported that the accumulation of Glu and Gln took place only in the tips of growing axes during the first 24 hours of imbibition at 18°C. The accumulated amount of Glu and Gln was enough to

account for increasing the growth potential of the embryonic axes. Since an increase in the osmotic potential of the embryonic axes is a necessary step for growth, it is necessary to determine precisely whether an increase in substances in the growing axes is the cause or the result of growth.

Glutamine was formed from Glu and ammonia by the action of glutamine synthetase (GS) in plant tissues. The activity of GS is not only affected by temperature but also has been demonstrated to be mediated by phytochrome (Takeba, 1980d). Both red light and gibberellin can increase the level of GS in seeds before the initiation of axis elongation. This increase is completely suppressed by cycloheximide (Takeba, 1983b, 1984). Sakamoto et al. (1990) demonstrated that red light increased the translatable mRNA for GS in lettuce seeds. It is assumed that GS is synthesized *de novo*.

Restriction of Lettuce Seed Germination at High Temperature

Generally at temperatures above 25°C, germination of lettuce seed is blocked because of thermoinhibition. Much research has been carried out on the nature of lettuce seed dormancy at high temperature and how to overcome it and the role of the outer seed coverings or embryo in restricting lettuce seed emergence at high temperature has been debated. One theory is that the endosperm layer restrict the radicle emergence. The endosperm cells may secrete cell-wall-degrading enzyme(s) to weaken the endosperm tissue, therefore allowing radicle emergence (Ikuma and Thimann, 1963b). Another suggests that the emerging radicle cannot develop enough thrust to overcome the mechanical resistance of the endosperm without first weakening the endosperm cell wall (Nabors and Lang, 1971a,b). Pavlista and Haber (1970) reported that germination of lettuce seed needed the mechanical force of the growing embryo pushing against the endosperm and the enzymatic weakening of the endosperm.

Embryonic Growth

The growth of the radicle and germination of seed at high temperature are not identical phenomena. Foard and Haber (1966) reported that localized expansion and mitosis occurred in thermodormant lettuce seeds, but did not contribute to the overall expansion of their embryo. From cytological studies of the lettuce embryo, Haber and Luippold (1960a,b) indicated that the occurrence of mitosis and cell elongation could be regulated physically and chemically. For example, the treatment with mannitol alone or a combination of kinetin and high temperature (37°C) caused mitosis prior to actual germination. However, at 37°C, when thiourea was present, it caused radicle protrusion to precede cell division.

Factors which prevent or promote germination are associated with the control of embryonic growth in lettuce seed. As temperature is raised to 30°C, germination is inhibited. Germination of lettuce seed also can be prevented at nonthermoinhibited temperature by placing them in an osmoticum such as mannitol (Kahn, 1960). However, if the endosperm is removed or punctured, the embryo itself can germinate at a high temperature or in osmotic restraint (Borthwick and Robbins, 1928; Scheibe and Lang, 1965). These results suggest that there are factors acting to prevent the ability of the embryo to develop sufficient force to penetrate the endosperm barrier.
Takeba and Matsubara (1979) measured the embryonic growth potential of 'New York' lettuce seeds and demonstrated that intact seeds could not germinate at 35°C because the growth potential was not enough to overcome the restraining force of the seed coat. At high temperature, metabolic activity that normally increases during germination might be restricted, therefore reducing the accumulation of osmotic constituents. For example, as temperature increased from 15° to 35°C, small fat bodies did not disappear in 'New York' lettuce seeds (Takeba and Matsubara, 1977). Moreover, the amount of amino acid accumulation was reduced, possibly related to the activity of GS. Takeba

(1983a,b) attempted to demonstrate that the activity of GS in 'New York' lettuce seeds was correlated to germination behavior at high temperature. High GS activity was detected in dry seeds, but this activity decreased rapidly during imbibition at 35°C, although the amount of GS protein did not change. The amount of ammonia increased abruptly during the early stages of imbibition at 35°C, suggesting blockage of ammonia-assimilation at high temperature. The GS activity decreased to a low level during the first 12 hours of imbibition at 35°C, but increased again during subsequent continued imbibition at low temperature (15°C) before breaking thermodormancy. Because the activity of GS in the embryonic axis was higher than that in the cotyledons, thermodormancy of 'New York' lettuce seeds may be related to the inactivation of GS during imbibition at high temperature.

Red light increased the growth potential of lettuce seed at 25° and 30°C but not at 35°C. At 25° and 30°C, the osmotic potential in red light-treated seeds decreased due to free amino acids. This decrease was sufficient to account for the changes in growth potential and enabled the embryo to overcome the resistance offered by the endosperm. At 35°C, the growth potential of seeds was far less than the restraining force of seed coats; this low growth potential was the cause of the thermodormancy (Scheibe and Lang, 1965; Scheibe and Lang, 1969; Takeba, 1980b,c).

<u>The Seed Coverings</u>

Lettuce seeds that have their endosperm and integument removed are released from thermoinhibition. The role of the seed coverings–the pericarp, integument, and endosperm–in restricting seed germination at high temperature needs to be further identified.

For imbibed seeds at high temperature, the greater delay and slower attainment of maximum germination suggests that metabolism inside the seed has been damaged

or essential metabolites for germination have been reduced to low levels (Gray, 1977). For instance, respiration in lettuce seed is inhibited at supraoptimal temperature because the endosperm and integumentary membrane prohibit the free diffusion of oxygen inward and carbon dioxide outward. As temperature increases, oxygen requirements rapidly increase, but the seed coat gradually becomes less permeable to gases so that the respiratory intensity is significantly decreased (Borthwick and Robbins, 1928).

ABA is an inhibitor of seed germination. Researchers have proposed that ABA inhibits lettuce seed germination at high temperature by inhibiting the synthesis of cell wall-weakening enzymes in the endosperm (Braun and Khan, 1975; Halmer and Bewley, 1979). Dulson et al. (1988) indicated that the amount of ABA that was leached from isolated endosperm correlated with mannanase production in isolated lettuce endosperm. McWha (1976) demonstrated the effect of temperature on ABA content in 'Great Lakes' seeds during imbibition. When the seeds were maintained at temperatures at which germination was delayed or prevented, the ABA level fell more slowly or not at all. However, Braun and Khan (1975) reported that the ABA content in germinating seed of 'Grand Rapids' diminished more rapidly at 25°C than at 35°C, but there was no relationship between ABA content and percentage of germination after imbibition for 24 hours. It is unknown what amounts of endogenous ABA are present specifically at active sites or are necessary to effect control of germination.

Dutta et al. (1994) used autohydrolysis to analyze the composition of cell walls. The results indicated that the autolytic activity of the isolated wall was related to germination conditions. In 'Pacific' lettuce seeds imbibing at 25°C, the rate of autolysis from the endosperm wall increased markedly in the period just prior to radicle emergence. The rate of autolysis at 32°C was reduced about 25% compared to that at 25°C. Seeds treated with ABA at 25°C did not germinate. The rate of autolysis from the endosperm cell walls of the seeds treated with ABA was significantly lower than that of

the seeds imbibed in water. Therefore, the capacity for lettuce seed germination at high temperature may be related to the activity of endosperm-cell-wall-degrading enzymes prior to radicle emergence.

The seed coverings act as a physical barrier in the restriction of lettuce seed germination at high temperature. Researchers measured the force required to puncture the different seed tissues by using an Instron Universal Testing Machine and tried to identify which part of the seed coverings played a major role in controlling germination (Drew and Brocklehurst, 1984; Tao and Khan, 1979; Wurr et al., 1987). In 'Grand Rapids', the major barrier to embryo growth was found to be the endosperm layer, which contributed 60% of the total resistance to puncture of the intact seed (Tao and Khan, 1979); but in 'Cobham Green', this value was only 40% (Drew and Brocklehurst, 1984). Wurr et al. (1987) as well as Drew and Brocklehurst (1990) measured the forces required to penetrate seed layers of different thermosensitive cultivars. The results indicated that the strength of the pericarp played a more important role than did the endosperm in determining germination. There were significant positive correlations between seed weight and the force required to penetrate the whole seed, but only one test cultivar had significant correlations between germination at high temperature and seed penetration forces. The results of the puncture test were not consistent, due possibly to the fact that different cultivars of lettuce seeds were used or the positions of penetration of achenes varied. Measurements of seed layer strength must accurately determine the force required to penetrate where the radicle protrudes into the seed coverings. Then, data of the penetration force would be more meaningful.

Increasing Thermotolerance in Lettuce Seed during Seed Production

Genotype

Lettuce is a salad crop that has been cultivated for several thousand years. Seeds of different cultivars vary in their germination in response to high temperature (Harrington and Thompson, 1952). Thompson et al. (1979) tested the germination of seeds of 23 cultivars of lettuce suitable for various cultivation seasons and reported that there were significant differences at the upper limit of temperature tolerance among various cultivars. However, no correlations were established in germination response, heading type, or growing season.

Gray (1975) studied 22 varieties of crisphead, cos, and butterhead types of lettuce and observed the response of seed germination for temperatures ranging from 5° to 33°C. The optimum temperature for germination in all varieties was between 15° and 22°C, and there was a noticeable upper temperature limit for germination that ranged from 25.7° to 32.8°C. Generally, seeds of the crisphead type germinated well at 30°C, a temperature which inhibited germination in the butterhead types.

Damania (1986) examined 62 genotypes of lettuce germplasm obtained from different countries and reported that the upper temperature limits for germination for most varieties ranged from 29° to 30°C. Although the results revealed that differences in the upper temperature limits for germination between varieties really existed, suggesting the possibility of improving the ability of seeds to germinate at high temperature by breeding (Damania, 1986; Gray, 1975), it is not yet clearly understood how seeds inherit the ability to germinate at high temperature.

It is interesting to note the possible correlation between the color of the seed coat and its ability to germinate at high temperature. Thompson et al. (1979) revealed that the surface characteristics of black and white achene were different. In black

achenes, the longitudinal ridges were more protuberant, more highly sculpted, had more transverse rows, and had better developed trichones than the white ones. Their studies of 23 lettuce cultivars revealed no significant correlation between the achene color and the germination response, although Damania (1986) studied 62 genotypes of lettuce germplasm and reported that black coated seeds tended to have lower temperature limits for germination than their white counterparts. Such contradictory results fail to make clear whether or not the structural differences in seed coats are related to seed germination performance at high temperature.

Temperature and Light

The geographic region where lettuce seeds are grown significantly affects the performance of seeds over a range of germination (Harrington and Thompson, 1952). Herringbone and Thompson (1952) studied the germination performance of 80 samples of seed representative of 12 lettuce cultivars and nine growing areas. The results indicated that the region where the seeds were grown had a significant effect on the ability of the seeds to germinate at high temperature; the seeds collected from hot climatic zones tended toward higher temperature tolerance limits for seed germination.

Thompson et al. (1979) pointed out that the level of temperature tolerance of the seeds was influenced by post-harvest maturity of the achenes as well as environmental conditions including temperature, light, aeration, and moisture tension under which the crop was grown and harvested. Gray et al. (1988b) studied the effect of temperature on seed germination of 'Saladin' crisp lettuce at 30°C and found that seeds produced at 30/20°C germinated more readily than those produced at 25/15°C or 20/10°C. Koller (1962) indicated that temperature and photoperiod conditions to which the ripening seed was exposed indeed affected the seed's subsequent germination behavior. When 'Grand Rapids' seeds maturated under high temperature (30/23°C), high-temperature

tolerance (26°C) increased. In addition, comparing the effect of diurnal (23/17°C) and constant (23° or 17°C) thermoperiods on seed germination at high temperature suggested that seed maturation under constant temperature may induce greater germination at high temperature than seed maturation under varying temperatures.

The photoperiod encountered during seed maturation is another factor that can affect the ability of seed to germinate at high temperature. Continuous light during seed maturation increased germination in comparison to seeds that matured under 8 hours of light per day (Thompson et al., 1979). The results of studies involving the effects of temperature and photoperiod on seed germination have led researchers to postulate that environmental factors during seed production can affect the level of thermotolerance of lettuce seed (Thompson et al., 1979).

Seed Development and Maturation

The quality of lettuce seeds harvested from mother plants may vary, even when the mother plants are grown in the same area and in the same cropping season. The lettuce inflorescence has a cymose cluster of flowers heads, with the oldest flowers at the terminal end of the main axis (Jones, 1927). Flowers open for no more than two hours. Pollination and fertilization occur in less than six hours and seeds are mature 12 days after pollination. Throughout the flowering period, which lasts about two months, the rate of flowering and seed maturation is regulated by temperature (Jones, 1927). Mother plants are thus exposed to varying air temperatures during the period of flowering. The temperatures during seed production affect seed quality; therefore, the quality of all the seeds produced through the season can vary (Damania, 1986).

Gray et al. (1988b) studied the effect of temperature on lettuce seed development, yield, germination, and seedling vigor. Seeds of 'Saladin' produced at 30°/20°C germinated better at 30°C than those matured at 25°/15° or 20/10°C. Seed

maturation temperature influenced the number of mature florets per plant, seed per floret, and mean seed weight. At 20°/10°C and 25°/15°C growth temperatures, seeds matured slowly but, compared to seed matured at 30°/20°C, seed dry weight and seed size were increased to 50% and 15%, respectively. The number of seeds and seed yield per plant increased with an increase in temperature from 20°/10°C to 25°/15°C but then declined with a further increase to 30°/20°C. Root length of seedlings was not affected by the seed maturation temperature.

Steiner and Opoku-Boateng (1991) investigated the effects of variation in ambient air temperature on 'Salinas' lettuce seed production. Mother plants were exposed to a wide range of temperatures during their flowering period in Fresno, California. Minimum temperatures during the day ranged from 11° to 22°C and the maximum ranged from 30° to 40°C. Seed size, weight, and yield were reduced, but germination percentage of those seeds increased with increasing minimum and maximum temperatures. Therefore, high temperature during seed development enabled the seed to subsequently germinate better at high temperature, but yield, in terms of total seed weight and/or total number of seeds, was not optimal, and seed size was reduced (Drew and Brocklehurst, 1990; Gray et al., 1988b). Environmental conditions that optimize yield and quality of lettuce seed and that ultimately lead to more thermotolerant seed need further investigation.

Different environmental factors affect seed quality in various ways. The chemical composition of a seed can be influenced by its position on the plant, the environment under which the parent plant is grown, and the management of cultural practices. Temperature during seed maturation and development affects the seed's chemical composition, including oil quality and protein concentration, both of which have been investigated in food and fiber crops (Fenner, 1992). The fatty acid composition of a number of seed oils has been reported to vary with the temperature under which the seeds have developed, with lower temperatures favoring increased unsaturated oils

(Miquel and Browse, 1995). In sunflower, the di-unsaturated linoleic acid (18:2) and the mono-unsaturated oleic acid (18:1) had equal proportions when seeds matured at high temperature (about 28°C). This ratio, however, was 6:1 when seeds matured at low temperature (around 12°C) (Harris et al., 1980). Temperature also affects the protein concentration in seeds of some species. Higher protein concentration at high temperatures may simply be the result of a reduction in carbohydrate accumulation or oil content (Correll et al., 1994; Leffel, 1988).

In lettuce seed, major stored reserves are lipids and proteins. How high temperature is associated with a change of oil or protein composition or both and the relationship between this change and thermotolerance of seeds is still unknown.

Seed Storage

In seeds of good quality, seedlings will emerge rapidly and uniformly and will be capable of establishing and developing under field conditions. Seed quality depends on the seed's genetic constitution, the environmental conditions prior to maturation and throughout the post maturation phase including harvesting, processing, and storage (Maguire, 1977).

In lettuce, the inflorescence is a spike on which the mature seeds are at the bottom of the inflorescence and the immature ones are at the top. When seeds are harvested, they are collected as one group exhibiting varying degrees of seed maturity (Damania, 1986).

Freshly-harvested lettuce seeds must be after-ripened for optimum germination, and cannot germinate at high temperature (Gray, 1975). This may be due to the need for anatomical and morphological changes in an immature embryo or to changes in chemical content of seeds during the after-ripening process (Mayer and Poljakoff-Mauber, 1989). The length of time lettuce seeds are stored after harvest affects their

response to germination temperature. Suzuki (1981) studied the effects of after-ripening on germination of lettuce. The physiological state of 'Grand Rapids' lettuce seed after harvesting was divided into three distinct stages: dormant, nondormant, and deteriorating. Fresh seeds were in the dormant stage and germinated well only in a narrow range of temperatures; however, this range gradually widened as a result of diminishing dormancy during after-ripening. Seeds which were after-ripened for 5 to 24 months had an increased germination at high temperature.

Seed vigor gradually increased with an increase in the length of storage after harvest and reached its maximum after 260 days of storage (Borthwick and Robbins, 1928; Gray et al., 1988b). After one year of storage, lettuce seeds are generally in a stage of deterioration. The physiological changes of seed deterioration can be demonstrated in seed color, delayed germination, and decreased tolerance to suboptimal environmental conditions (Suzuki, 1981).

Release of Thermodormancy by Ethylene and Cytokinins

Induction of dormancy in lettuce seeds be prevented by GA application or irradiation; however, seeds still require other hormones during germination for alleviation of high temperature dormancy (Khan, 1980/81). Cytokinins, ethylene, and GAs, alone or in combination, have been involved in alleviating the effect of high temperature (Braun and Khan, 1976; Dunlap and Morgan, 1977a; Hegarty and Ross, 1979; Keys et al., 1975; Rao et al., 1975; Sharples, 1973). Braun and Khan (1976) indicated that 'Mesa 659' (light-insensitive) and 'Grand Rapids' (light-sensitive) seeds could overcome thermodormancy by a complex of GAs, ethylene, and kinetin. Along with certain combinations of these hormones, GAs were able to release thermodormancy in 'Grand Rapids' seeds at 32°C. However, the action of GAs was not as effective in relieving thermodormancy of 'Mesa 659' seed at 32°C. At 35°C, the most effective relief for

thermodormancy of these two varieties was a combination of GAs plus kinetin and ethylene. In addition, CO_2 has been reported to enhance the stimulating effect of growth regulators in relieving thermodormancy (Key et al., 1975; Negm et al, 1972; Saini et al., 1986)

The effects of ethylene or cytokinins during early imbibition are different. Smith et al. (1968) dipped dry lettuce seed in kinetin solution for three minutes. Kinetin appeared to act at a very early stage of germination to relieve thermodormancy. Imbibing the seeds for various periods of time did not change the response to the dip treatment. In the early imbibition phase, the presence of ethylene was not required to release thermodormancy. Fu and Yang (1983) indicated that lettuce seeds did not respond to exogenous ethylene during the first 12 hours of imbibition. The effect of ethylene on releasing dormancy was exerted during the imbibitional phase immediately before the emergence of the radicle, and the maximum effect of ethylene was exerted between 24 and 36 hours of imbibition. These results suggest that, in overcoming thermodormancy, ethylene and cytokinins have different action sites.

Both ethylene and kinetin can increase the growth of the embryonic hypocotyl (Abeles, 1986; Takeba and Matsubara, 1979). Kinetin is a more effective promoter than ethylene for reversing induced dormancy (Dunlap and Morgan, 1977a). Kinetin acts to stimulate cotyledon expansion or hypocotyl longitudinal expansion or both (Abeles, 1986; Ikuma and Thimann, 1963a). Ethylene acts to control germination by promoting radicle expansion of the hypocotyl. However, the longitudinal expansion of the hypocotyl is more effective in initiating germination than the radial expansion of the hypocotyl (Abeles, 1986). Kinetin also strongly inhibits the growth of roots. Therefore, kinetin induces atypical germination, with cotyledon protrusion, a stunted radicle, or both. Once a wide opening is made at the cotyledon end of the endosperm coat, the embryo can slip through this opening. This presumably means that the hypocotyl can be a source of pressure to drive the radicle through the restraining endosperm. Root

growth does not appear to be required for germination since it is normally initiated after the radicle penetrates the endosperm (Braun and Khan, 1976; Dunlap and Morgan, 1977a; Ikuma and Thimann, 1963a).

There are two distinctly different rates of ethylene production during germination of lettuce seeds. Dunlap and Morgan (1977b) reported a very low rate of ethylene released prior to the twelfth hour of incubation at 22°C. The rate of ethylene production increased 100 fold between the twelfth and sixteenth hour of incubation, after which it decreased. Seeds did not germinate or produce ethylene when they were incubated at temperatures above 30°C (Negm et al., 1972; Saini et al., 1986). The effect of high temperature on ethylene production can be explained by changing the structure of the cell membrane and the structure of ethylene-forming enzyme (EFE). This enzyme, EFE, is essential for ethylene production and synthesizes ethylene from 1-aminocyclo-propane-1-carboxylic acid (ACC); it is a membrane-bound enzyme and is oxygen-dependent. Increasing the temperature to 35°C causes a decrease of EFE activity, accumulation of ACC, and reduction of ethylene production (Corbineau et al., 1988; Lieberman, 1979; Yang, 1985; Yu et al., 1980).

Small et al. (1993) reported that oxygen plus kinetin almost completely alleviated thermoinhibition in 'Grand Rapids' seeds. The results suggested that oxygen plus kinetin either caused seed to bypass an ethylene requirement for germination or increased the sensitivity of the seed to ethylene. In air at 38°C, seeds exhibited a high level of ethanolic fermentation. This was probably because of lower oxygen availability resulting from a decrease in oxygen solubility at this temperature. However, the seeds respired aerobically in a treatment of oxygen plus kinetin at 38°C. This treatment also increased the level of ATP in the seed which was able to satisfy the requirement for energy to drive germination processes at 38°C.

Prusinski and Khan (1990) slit lettuce seeds by making a longitudinal cut at the cotyledon end. This treatment enhanced seed germination above 32°C and increased

ethylene production. This enhancement could be attributed partly to the removal of the restraining force of the seed coat and partly to the increased conversion of ACC to ethylene. The effects of seed slitting on ethylene production and seed germination at high temperature depended on genotype. For example, in 'Mesa 659' and 'Super 59', slitting greatly enhanced ethylene production and germination; in 'Grand Rapids', it had minimal effect on them. The amount of ethylene produced during stress from various cultivars generally correlated with their ability to increase germination potential. Therefore, the ability of seed to produce ethylene under stress conditions may be used in screening stress-tolerant lettuce cultivars.

Seed Priming as a Means to Overcome Thermodormancy

Priming consists of imbibing seeds in an osmotic solution for a specific period of time at a certain temperature. The osmoticum is usually inorganic salts or polyethylene glycol (PEG) in water. The concentration of osmoticum must be adjusted to a level high enough to inhibit radicle protrusion, thereby permitting prolonged metabolic reactions during the lag phase of water uptake (Heydecker and Gibbins, 1978; Karssen et al., 1989). Successful priming depends on treatment duration, temperature, aeration, and the water potential of the priming solution; in addition, species, cultivar, seed quality, dehydration after priming, and seed storage conditions also affect the results (Guedes, 1979; Heydecker and Gibbins, 1978; Parera and Cantliffe, 1994).

Seed priming can shorten the period from sowing to seedling establishment and reduce the risk of seeds being exposed to adverse environmental and biotic factors in the field during this critical period (Khan et al., 1978). Bradford (1986) as well as Parera and Cantliffe (1994) detailed methods for successful osmotic priming in a variety of species, including carrot (*Daucus carota* L.), celery (*Apium graveolens* L.), tomato (*Lycopersicon esculentum* Mill), onion (*Allium cepa* L.), lettuce, and others. In several

varieties of lettuce, a variety of osmotica successfully release thermodormancy. For example, seeds of 'Valmaine' lettuce, which are sensitive to high temperature during germination, had significantly more germination rate at 30°C after seeds were primed in -5.1 bar K_3PO_4 + PEG (Cantliffe et al., 1981). Thermodormancy in seeds of 'Mesa 659' was overcome by priming for 2 weeks in a solution of -8.4 bar PEG at 16°C (Khan et al., 1980/81). Seeds of 'Minetto' were primed in an aerated solution of 1% K_3PO_4 at 15° C for 20 hours to bypass thermodormancy (Guedes and Cantliffe, 1980). These results indicated that thermodormancy in lettuce can be successfully overcome by proper priming.

Physiological and Biochemical Aspects of Seed Priming

Priming initiates several physiological and biochemical changes in lettuce seeds. For instance, it shortens the time before the onset of RNA and protein synthesis, increases the amount of RNA and protein synthesis, increases the activity of many enzymes, and accelerates the metabolic rate (Khan et al., 1978; Mayer, 1977).

DNA synthesis during the priming period is determined by using flow cytometry. The results indicated that the chromosomal material in root cells had ceased cell cycle activity at the G_2 phase, and that the action of replicative synthesis preceded germination in tomato and pepper (*Capsicum annuum* L.) embryos (Bino et al., 1992; Lanteri et al., 1993; Lanteri et al., 1994). However, primed seeds of leek (*Allium porrum* L.) and brussels sprouts (*Brassica oleracea* var. *gemmifera*) did not show DNA synthesis during the priming period; however, a DNA repair mechanism appeared to be functioning. According to these results, seed priming resulted in neither cell division nor S-phase DNA synthesis (Ashraf and Bray, 1993; Bray et al., 1989; Burgass and Powell, 1984; Clarke and James, 1991). Therefore, the differences in DNA synthesis in primed

seeds of a number of species may be a species-dependent variation, or may be caused by differences in experimental conditions.

The synthesis of DNA increases greatly in primed compared to nonprimed seeds. When primed pepper and leek seeds were subsequently reimbibed in water, the induction of DNA synthesis started earlier compared to untreated seeds (Bray et al., 1989; Lanteri et al., 1993). In lettuce seeds, the timing of DNA synthesis occurred just prior to radicle protrusion. Cotylenin E, a fungal toxin that promotes cell elongation but not cell division or DNA synthesis, delays the protrusion of the radicle of primed seed (Khan, 1980/81; Sessa et al., 1975). Therefore, the initial effect of priming may be largely on cell elongation rather than on cell division and DNA synthesis.

The amount of RNA synthesis in seeds is much greater than the amount of DNA synthesis during or immediately following seed priming (Coolbear and Grierson, 1979; Khan et al., 1978). The cellular RNA content in primed leek and tomato embryos increased dramatically through the synthesis and accumulation of rRNA (Coolbear and Grierson, 1979; Coolbear et al., 1990; Davison et al., 1991). Cordycepin, an inhibitor of RNA synthesis, strongly inhibited the germination of nonprimed but not primed lettuce seed. If cordycepin was added to the osmoticum during priming, the radicle protrusion in primed seed was not inhibited (Khan, 1980/81; Khan and Samimy, 1982; Tao and Khan, 1976). Therefore, RNA synthesis does not appear to be obligatory for radicle protrusion.

Several researchers have reported that there is an enhancement of protein synthesized in seeds in a number of crops during the priming period and subsequent germination (Bray et al., 1989; Dell'Aquilla and Tritto, 1989; Khan et al., 1978; Khan et al., 1980/81). Davison and Bray (1991) used two-dimensional polyacrylamide gel electrophoresis to analyze protein synthesis in leek seed. There were quantitative and qualitative differences in the protein synthesized in primed versus nonprimed seeds and during priming versus germination. During the priming period, five polypeptides were

specially synthesized in leek embryo and endosperm. However, the role of the priming-specific polypeptides was not clear. Khan and Samimy (1982) suggested that activation or synthesis of proteins or both in primed seeds may play a role in initial events concerned with cell expansion. In lettuce seed, protein synthesis is enhanced during or following priming (Khan et al., 1978). Cycloheximide, an inhibitor of protein synthesis, inhibits the rate of protein synthesis and early germination in primed and nonprimed seeds of lettuce (Khan et al., 1980/81). Khan et al. (1980/81) suggested that improvement of seed germination by seed priming may be influenced by an enhancement in activities of protein synthesis.

Enzyme and metabolic activity are enhanced during seed priming and are related to seed invigoration during germination (Khan et al., 1978). Khan et al. (1978) suggested that the activity of acid phosphatases and esterases in primed lettuce seeds increased as a result of activation or *de novo* synthesis. Enzyme data suggest that priming possibly affects key metabolic processes that are related to the breakdown of protein and lipid reserves (Khan et al., 1980/81). A large-scale mobilization of storage reserves occurs during priming in order to be able to provide substrates which are readily utilized for germination. Many of these small molecules evidently have osmotic properties that can decrease water potential. This degradation of stored reserves can increase the ability of the primed seeds to readily absorb water, to germinate rapidly, and to tolerate stressful environments (Khan, 1992).

Physiological studies have indicated that rates of metabolic processes involved in germination of primed seed can be increased. Mazor et al. (1984) indicated that ATP formation in pepper, spinach (*Spinacia oleracea* L.), eggplant (*Solanum melongena* L. var. *esculentum* Nees.), and kohlrabi (*Brassica oleracea* L. var. *gongylodes*) seed was increased during the priming period. Dehydration of primed seeds reduced ATP levels, but not to a point below those of nonprimed seeds. In an analysis of nucleotide and nucleotide sugar levels in leek embryos during priming and subsequent germination,

Bray et al. (1989) reported that only traces of GTP and CTP as well as low levels of ATP and UTP were present in embryos during priming. After a six-hour lag period following the end of priming, the levels of ATP and UTP increased sharply. The production of metabolic energy is required to synthesize macromolecules, membranes, and cell wall materials for germination. The biochemical changes in the primed seeds indicate that there is considerable biochemical activity during priming. This activity is attributed to the significant benefits in germination performance of primed seeds.

Mechanism of Priming in Overcoming Thermodormancy

Priming circumvents thermodormancy in lettuce by bypassing the critical germination period in which thermodormancy would normally be induced. A limited number of physiological and biochemical studies explain the improved germination at high temperature effected by priming, such as DNA and RNA metabolism, nucleotide, and nucleotide sugar levels, as well as changes in proteins and enzymes (Karssen et al., 1989). However, it still is not clear which factor(s) control the initiation of lettuce seed germination in overcoming thermodormancy.

In lettuce seed, germination is inhibited at supraoptimal temperature. Part of the mechanism of thermoinhibition could be due to the failure of the ATP content to reach a sufficient level to satisfy the requirement for germination at high temperature. Small et al. (1993) suggested that increased respiration and ATP production during priming may be the primary mechanisms in alleviating thermoinhibition. Researchers have reported that respiratory activity and the formation of ATP appeared to increase during seed priming in a number of species (Mazor et al., 1984; Smith and Cobb, 1989). However, in lettuce, when primed seed germinated at 35°C, respiration did not appear to be hindered and ATP production was not inhibited when compared with nonprimed seeds. These results indicated that seed priming did not markedly alter the pattern of

respiration (Cantliffe, 1976; Cantliffe et al., 1984). Therefore, to understand the role of metabolic changes during seed priming and subsequent germination, more detailed experiments are needed to elucidate possible mechanisms in the relief of thermodormancy.

Cantliffe et al. (1984) suggested that the osmotic potential of the lettuce radicle should be increased so that cell elongation could occur, since this process was inhibited by high temperature. In primed lettuce seeds, increased accumulation of soluble amino nitrogen compounds and other hydrolytic products in the radicle tips could be a mechanism for overcoming thermodormancy. Researchers (Georghiou et al., 1983; Psaras et al., 1981) indicated that, in the germinating seed, the endosperm cells opposing the radicle were highly vacuolated and storage materials were mobilized prior to radicle protrusion. However, the endosperm cells at the lateral and cotyledonary end remained unchanged. Khan (1980/81) pointed out that the rate, quality, and quantity of enzyme synthesis were influenced by priming. Data on enzymatic changes suggested that priming probably affected key metabolic processes related to the breakdown of protein and lipid reserves as well as to glycolysis and the turnover of phosphate esters. However, Weges et al. (1991) suggested that changes in the level of dormancy in lettuce seeds occurred independently from soluble amino nitrogen metabolism and osmotic potential. To delay relief of dormancy, they primed seeds in PEG at 15°C, but this priming did not prevent a continued rise in the activity of glutamine synthetase. Therefore, it is possible that the accumulation of soluble amino nitrogen compounds occurring during priming plays some other beneficial role.

Karssen et al. (1989) suggested that osmotic priming facilitated a quick and uniform germination by stimulating cell wall extensibility in the radicle and weakening the endosperm cell walls. In tomato seeds, the embryo had expanded growth prior to the emergence of the radicle. The endosperm tissue enclosing the embryo restricted further hydration until weakening of its cell walls occurred so as to permit radicle

emergence. Priming of tomato seeds may lead to more rapid germination by modifying these mechanisms. For example, a rapid expansion of embryo from primed seed was attributed to changing the extensibility in the radicle cell wall during priming (Groot and Karssen, 1987). The 50% reduction in germination time was a function of the reduction of the mechanical resistance of the endosperm tissue in primed seed. In lettuce seeds, Karssen et al. (1989) mentioned that a comparison of cultivars indicated that the deeper the dormancy, the larger the effect of the enclosing structures on restraining radicle protrusion. Guedes et al. (1981) viewed morphological changes in 'Minetto' seeds during priming with a scanning electron microscope and reported that the membranes of endosperm cells were gradually loosened after nine hours of priming. This loosening may be symptomatic of membrane weakening and possibly is one of the priming mechanisms involved in enhancing seed germination at high temperature.

In summary, the general benefits of priming are substantial. Under unfavorable environmental conditions, germination of primed seeds is faster and more uniform. The physiological and biochemical changes that take place during priming have been investigated in a number of crops and vary with genotype. In lettuce seed, the mechanism of priming that results in increased embryonic growth or weakening of the endosperm is still unknown.

By manipulating the environment in which the seeds are produced, thermotolerance may be enhanced, especially in certain more thermotolerant genotype. To that end, the primary objectives of this study were to use the various ways of relieving thermoinhibition in lettuce seeds, such as seed priming, puncture test to measure seed covering resistance, and morphological ultrastructural observations, and to identify restrictions to lettuce seed germination at supraoptimal temperature.

CHAPTER 3
SEED DEVELOPMENTAL TEMPERATURE REGULATION OF THERMOTOLERANCE IN LETTUCE

Introduction

The primary method of planting lettuce (*Lactuca sativa* L.) commercially is by direct seeding. Lettuce seed germination is strongly temperature dependent, with the optimum temperature range for seed germination in most lettuce cultivars in a range between 15° and 22°C with the upper limit ranging from 26° to 33°C, depending on genotype (Gray, 1975). When temperature rises 2° to 3°C above the maximum level for a specific genotype, germination sharply declines. This phenomenon is termed thermoinhibition. Subsequently, if the temperature is quickly lowered, this inhibition can be reversed and germination proceeds. However, if the imbibed seeds are subjected to high temperature for an extended period of time, a secondary dormancy, called thermodormancy, is induced. Such dormant seeds cannot germinate even if they are maintained at low temperature (Khan, 1980/81).

To avoid poor stands when lettuce is planted in the field under conditions of high temperature, it is necessary to bypass or release thermoinhibition in the seed before planting. Thermoinhibition is a more transient condition than thermodormancy, so preventing thermoinhibition blocks the onset of thermodormancy.

The level of thermotolerance and its effects on lettuce seed germination are genotype-dependent (Gray, 1975; Thompson et al., 1979). A number of lettuce genotypes that germinate at high temperature have been identified. One of these, PI 251245, is particularly thermotolerant (Bradford, 1985). The Spanish bibb type lettuce 'Maturo' has also been widely used as parental material in many breeding programs. It is believed that 'Maturo' transfers its thermotolerant character to other cultivars such as 'Tall Guzmaine',

'Everglades', and 'Floricos 83' (Guzman, 1986; Guzman et al., 1992; Guzman and Zitter, 1983).

Both light and the environment in which the seed matures can affect germination performance at superoptimal temperature. Photosensitive lettuce seed has a functional phytochrome system and is affected in its germination responses by red or far-red light irradiation (Borthwich et al., 1952). Evenari et al. (1953) have pointed out that there are two possible photo-mechanisms influencing germination. One mechanism ensures that germination is not affected by light and operates down to a certain temperature limit. Below that limit germination can not be decreased by light alone. The other mechanism is light-sensitive, responding to the effects of red as well as far-red light and operates up to the temperature limit above which germination cannot be increased by light alone.

The upper temperature limit can be modified by manipulating the environment during lettuce seed production (Harrington and Thompson, 1952). Damania (1986) examined 62 genotypes of lettuce obtained from different countries and indicated that seeds collected from hot climatic zones tended to germinate at higher temperatures. Moreover, seed maturation at high temperature can affect the seed's subsequent germination behavior (Gray et al., 1988b; Koller, 1962). Seed germination percentage at high temperature can be increased, but seed size, weight, and yield may be reduced (Drew and Brocklehurst, 1990; Steiner and Opoku-Boateng, 1991).

The objective of this investigation was to determine if temperature under which the seed developed could alter the level of thermotolerance at germination in various thermosensitive lettuce genotypes. By applying a range of developmental temperatures during seed development, it may be possible to precisely define the trigger needed to improve germination at formerly superoptimal temperatures.

Materials and Methods

Selection of Germplasm

Seed of 21 genetic lines known to display various levels of thermotolerance were obtained through the breeding program of R.T. Nagata, University of Florida, Everglades Research and Education Center, Belle Glade. In a preliminary test, plants from the 21 lines were grown and, as the flower buds became visible, 20 plants from each line were moved into growth chambers (E-15, Conviron) that maintained temperatures of 20°/10°, 25°/15°, 30°/20°, and 35°/25°C (day/night) in a 12 hour photoperiod (500 μmol m^{-2} s^{-1}) and 75-80% RH. As the plants grew, they were irrigated daily and were fertilized as needed with 3500ppm of 20N:8.6P:16.7K. Mature seeds were harvested from plants growing in each growth chamber, then stored for two months to after-ripen.

Preliminary Germination Tests

Genotypes were selected according to their ability to germinate under high temperature. Germination tests were conducted on a one-dimensional thermogradient bar (Type DB 5000, Van Dok & De Boer B.V.) in a linear temperature gradient of 20°, 24°, 27°, 30°, 33°, 35°, and 38°C.

Twenty seeds from each genotype, matured at each of the four temperatures, were placed on 5 cm diameter blue blotters (Anchor steel blue seed germination blotters) moistened with 6 ml of distilled water and positioned at areas of the bar set at 20°, 24°, 27°, and 30°C. In order to maintain constant moisture at temperatures of 33°, 35°, and 38°C, two layers of blotter paper were used and the paper was initially moistened with 8 ml of distilled water. Blotters were covered with 5.5 cm Petri dish lids and were remoistened with distilled water as needed. Germination was in the dark and was documented when a visible radicle protruded through the seed coat. All treatments were replicated three times.

Seeds of an additional 10 lettuce genotypes produced in the Salinas area of California in 1992 were germinated. Germination tests, replicated three times for each genotype, were conducted at temperatures of 20°, 24°, 27°, 30°, 33°, 35°, and 38°C.

Selection of Various Thermosensitive Genotypes

Various genotypes were selected according to their germination potential and classified as thermosensitive ('Valmaine' and 'Dark Green Boston', Table A-1) or thermotolerant ('Floricos 83', Table A-1; 'Everglades' and PI 251245, Table A-2).

'PI 251245' is a wild type. 'Everglades' and 'Dark Green Boston' are butterhead types. 'Floricos 83' and 'Valmaine' are cos types.

Seed Production

Seedlings of selected genotypes were cultivated in 15 cm diameter pots containing media consisting of a mixture of coarse vermiculite and Speedling Fortified Tobacco Mix® (1:5 v/v). The pots were placed in a greenhouse located in Gainesville, Florida. In the greenhouse, the average day/night temperature in summer was 40°/28°C and in winter 25°/15°C, light was about 550 μmol m^{-2} s^{-1} and RH was 75-80%. As the plants grew, they were irrigated daily as needed and were fertilized once every 14 days using 3500 mg $liter^{-1}$ of 20N:8.6P:16.7K. As the flower buds became visible, 20 plants from each genotype were moved to each of four growth chambers, for a total of 80 plants per genotype. All growth chambers were programmed for a 12 hours photoperiod, a light intensity 500 μmol m^{-2} s^{-1} and a RH of 75-80%. Day/night temperatures were 20°/10°, 25°/15°, 30°/20°, or 35°/25°C. Seed production was repeated in May, 1993, January, 1994 and June, 1994.

Pollination occurred naturally in all genotypes at all temperatures except 35°/25°C and for 'Everglades' at 30°/20°C which required hand-pollination by means of cotton swabs (Johnson & Johnson). When seeds matured, they were harvested by hand, bulked,

threshed, and cleaned. Seeds were stored at 10°C and 45% RH for six months to after-ripen. After storage, 100 seeds of each genotype and treatment were weighed out and the shapes were recorded by using a Hewlett-Packard ScanJet 3C/T scanner.

Germination Test

Germination temperatures on the thermogradient bar were 24°, 27°, 30°, 33°, and 36°C. Thirty seeds of each genotype and each seed maturation temperature were placed on one layer of 5 cm diameter blotter paper and moistened with distilled water. Two layers of blotter paper were used for the 33° and 36°C treatments. Germination tests were conducted under florescent light (~26 μmol m^{-2} s^{-1}) in a 12 hour photoperiod or in the dark. Germination was measured as radicle protrusion and was recorded daily for 7 days. Germinated seeds were removed after they were counted. Total percent germination and mean days to germination (MDG) were calculated as a measure of reponse to each treatment. The MDG was calculated according to the formula $\sum$Ti Ni / $\sum$Ni, where Ni is the number of newly germinated seed at day Ti (Maguire, 1962).

Experimental Design and Statistical Analysis

Two factors were evaluated in the germination tests: genotype and seed maturation temperature. Five genotypes and four seed maturation temperatures were evaluated. The study was conducted using a split-block experimental design. Germination temperature was assigned to the main block and seed lots or treatments as the split block. The experiment was replicated three times.

Germination percentages were transformed to a square root arc sine basis prior to statistical analysis. Analysis of variance (ANOVA) of data was performed by means of Statistical Analysis System (SAS) software (SAS, 1987). The effect of germination temperature was partitioned into linear, quadratic, or cubic orthogonal contrasts. The effect

of seed maturation temperature was partitioned using a single degree of freedom orthogonal contrast. The first contrast compared the response between the lower temperatures (20°/10°C and 25°/15°C) and the higher temperatures (30°/20°C and 35°/25°C), the second contrast tested the response within the lower temperatures, and the third contrast tested the response within the higher temperatures. Means were separated by the Least Significant Difference (LSD) test.

Results

Effect of Light on Seed Germination

All seeds that were produced in the Salinas area in California germinated at 24°C, regardless of genotype or light condition (Table 3-1). At 27°C, germination of 'Dark Green Boston' (a thermosensitive genotype) was 74% in light, but did not germinate in the dark or at any temperature above this. Germination of 'Valmaine' (a thermosensitive genotype) was partially inhibited in light at 30°C and seed did not germinate above 27°C or in the dark. 'Floricos 83', 'Everglades', and PI 251245, all thermotolerant genotypes, germinated at 30°C in light or darkness. Above 30°C, germination of 'Everglades' declined both in light and darkness. For 'Floricos 83' and PI 251245, germination in light at 33°C was 83% and 94%, respectively, but fell to 40 to 50% in the dark. At 36°C, the PI 251245 germinated in light.

Seed Weight and Shape

When seeds were maturated at temperatures ranging from 20°/10°, 25°/15°, 30°/20°, to 35°/25°C, seed weight of all five genotypes decreased (Table 3-2). Seeds matured at 20°/10°C and 25°/15°C were significantly heavier than those matured at 35°/25°C. Seed weights were 37% and 22% greater at the lower temperature regime for 'Dark Green Boston'

Table 3-1. Germination percentage of lettuce seeds germinated over a temperature range of 24° to 36°C in 12h light/12h dark or dark.

	Dark Green Boston		Everglades		Floricos 83		Valmaine		PI 251245	
°C	L/D	D	L/D	D	L/D	D	L/D	D	L/D	D
					(%)					
24	100	100	100	100	100	100	100	100	100	100
27	74.0	0	100	100	100	100	100	100	100	100
30	3.3	0	100	100	99.0	97.7	76.0	18.0	100	100
33	0	0	43.0	37.0	83.0	41.0	4.3	0.0	94.0	52.0
36	0	0	6.0	3.3	4.7	0.0	0.0	0.0	30.7	17.7
Temp	**[z]		**		**		**		**	
Light	**		NS		**		**		**	
Temp x Light	**		NS		**		**		**	
Temp x Light $LSD_{(0.05)}$=	11.1				9.1		6.0		12.8	

[z]NS, *, ** Nonsignificant or significant at P=0.05 or 0.01, respectively, by F-test.

Table 3-2. Weight (mg/ 100seeds) of lettuce seeds matured under four temperatures.

	Seed maturation temperature (day/night, °C)				
Genotype	20/10	25/15	30/20	35/25	$LSD_{(0.05)}$
Dark Green Boston	169[z]	146	125	107	13
Everglades	171	163	143	133	6
Valmaine	146	147	142	103	31
Floricos 83	156	138	125	107	22
PI 251245	217	155	160	145	10

[z] Mean separation within genotypes by Least Significant Difference test at P=0.05.

and 'Everglades', respectively. In the other three genotypes, weight was about 30% to 33% more at the lower seed maturation temperatures.

Shapes and relative seed sizes of all five genotypes matured under the four temperatures are shown in Figure 3-1. Among them, seeds that matured at 20°/10°C and 25°/15°C were larger than in size those matured at 35°/25°C.

Effect of Seed Maturation Temperatures on Seed Germination

The main effects of germination temperature and seed maturation temperature accounted for most of the variance explained by the statistical model in analyzing germination percentage (Appendix, Table 3). Interactions between seed germination temperature and seed maturation temperature were statistically significant. Basically, the temperature at which seeds germinated had an inverse relationship to percentage germination: wherein as temperature increased, percentage germination decreased. Seeds that matured at or above 30°/20°C had a greater capacity to germinate at high temperature.

The upper temperature limit for germination of 'Dark Green Boston' was significantly enhanced when the seeds matured at 30°/20° and 35°/25°C (Figure 3-2). When seeds matured at 30°/20°C, the percentage of germination was 54% at 36°C, while those seeds matured at 25°/15°C had greater germination at 27°C than seeds matured at 20°/10°C. Seeds from plants grown at 20°/10° and 25°/15°C essentially did not germinate above 27°C.When 'Everglades' seeds were germinated at 33° and 36°C, seeds matured at 30°/20°C had better germination than those matured at 35°/25°C (Figure 3-3). At 33° and 36°C, seeds matured at 30°/20°C and 35°/25°C had higher germination percent than those matured at 20°/10° and 25°/15°C.

At 27°C, all seeds of 'Valmaine' germinated similarly regardless of maturation temperature (Figure 3-4). Seeds matured at 20°/10°C had less than 50% germination at 30°C while germination was unaffected in seeds matured at the other three temperature

Figure 3-1. Compare of shape of lettuce seeds matured under four temperatures.

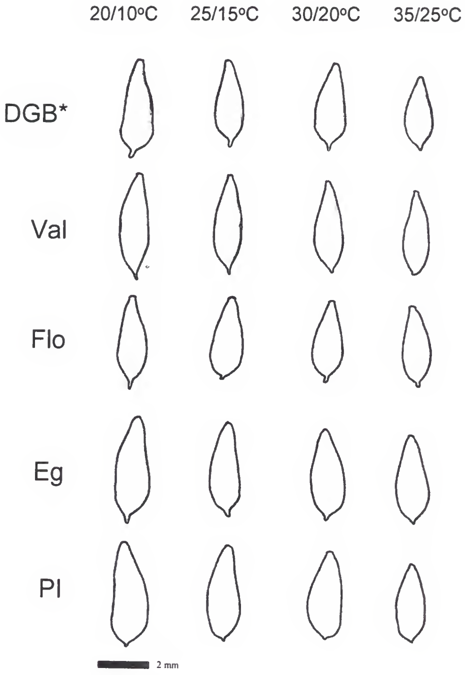
* DGB: Dark Green Boston

  Val: Valmaine

  Flo: Floricos 83

  Eg: Everglades

  PI: PI 251245


20/10ºC
25/15ºC
30/20ºC
35/25ºC
DGB*
Val
Flo
Eg
Pl
2 mm

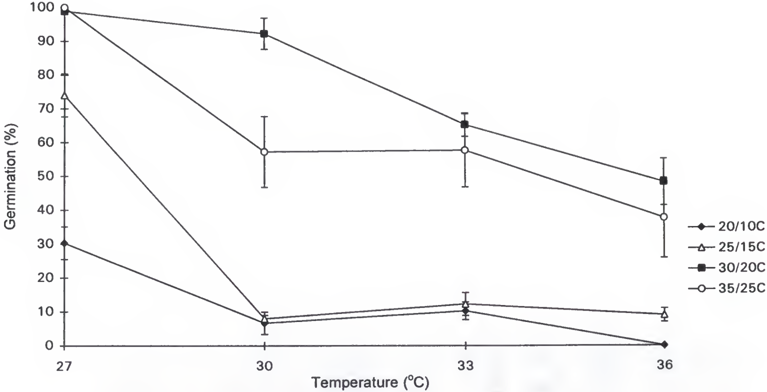


Figure 3-2. Germination percentage of 'Dark Green Boston' lettuce seeds matured under four temperatures in 12h light/12h dark. Vertical bars indicate standard error.

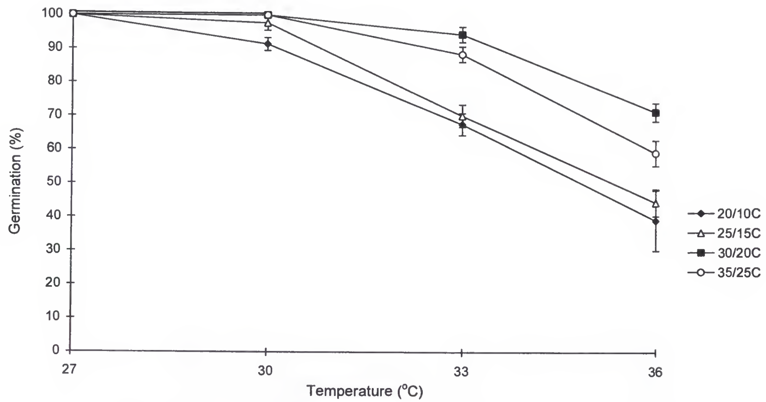


Figure 3-3. Germination percentage of 'Everglades' lettuce seeds matured under four temperatures in 12h light/12h dark. Vertical bars indicate standard error.

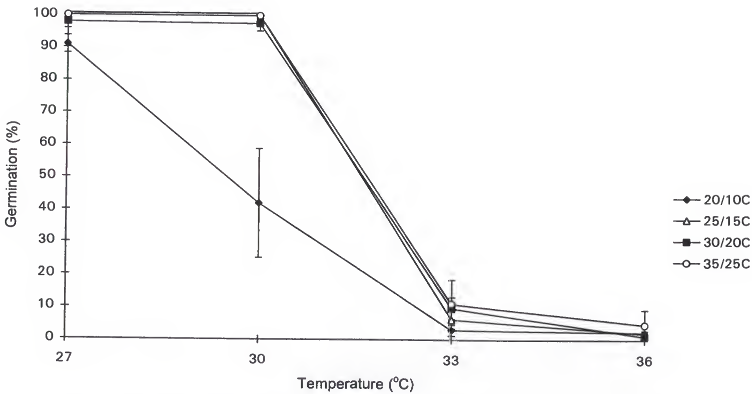


Figure 3-4. Germination percentage of 'Valmaine' lettuce seeds matured under four temperatures in 12h light/12h dark. Vertical bars indicate standard error.

regimes. 'Valmaine' essentially did not germinate above 30°C, regardless of maturation temperature.

Seeds of 'Floricos 83' matured at 35°/25°C and 30°/20°C had more than 50% germination 36°C (Figure 3-5). At 33°C and 36°C, germination of seeds matured at 20°/10°C and 25°/15°C was below 40%.

Seeds of PI 251245 matured at 35°/25°C had nearly 100% germination at 36°C (Figure 3-6). Seeds matured at 30°/20°C and 25°/15°C had excellent germination at 36°C. Seeds matured at 20°/10°C had a linear decrease in germination as temperature increased from 27°C to 30°C. Germination at 36°C of seeds from this maturation temperature was significantly less than that of seeds produced under the other temperatures.

For all genotypes, average mean days to germination increased as temperature increased from 27°C to 36°C, regardless of maturation temperature (Tables 3-3 to 3-7). With the exception of two isolated cases for 'Floricos 83' and PI 251245, there were no significant interactions between seed maturation and germination temperature (Appendix, Table 4).

Other than 'Valmaine', the genotypes generally germinated more rapidly when seeds matured at the lower temperatures (20°/10°C and 25°/15°C) rather than at the higher temperatures (30°/20°C and 35°/25°C).

Discussion

Five genotypes were selected from lettuce germplasm for their varying thermotolerance: 'Dark Green Boston' and 'Valmaine' were grouped as thermosensitive, while 'Floricos 83', 'Everglades', and PI 251245 were grouped as thermotolerant. Despite the limited information available on the inheritance of thermotolerance, the results from these and other studies indicated that real genotypic differences existed in the upper temperature limit for germination (Damania, 1986; Gray, 1975).

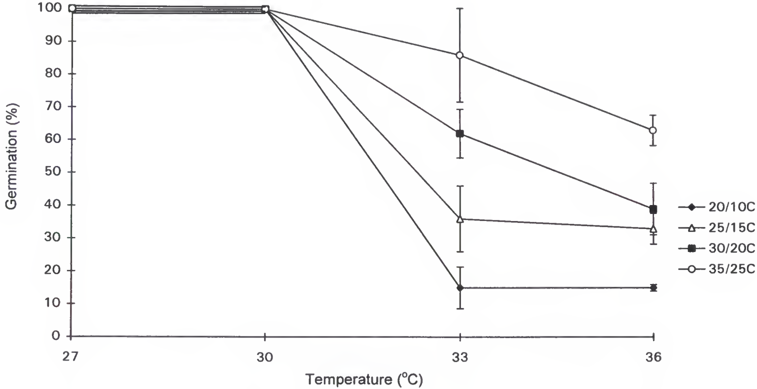


Figure 3-5. Germination percentage of 'Floricos 83' lettuce seeds matured under four temperatures in 12h light/12 h dark. Vertical bars indicate standard error.

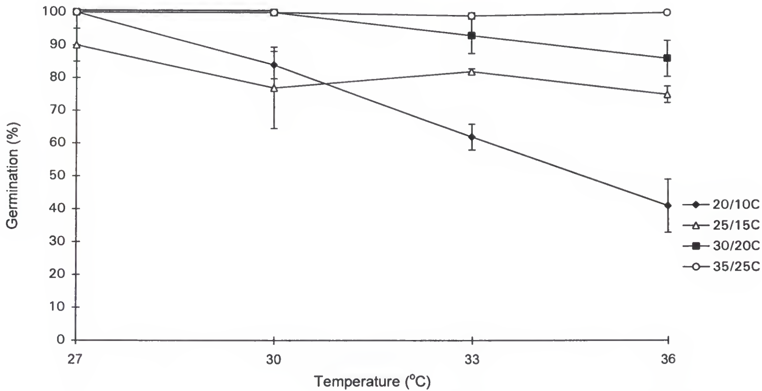


Figure 3-6. Germination percentage of PI 251245 lettuce seeds matured under four temperatures in 12h light/12h dark. Vertical bars indicate standard error.

Table 3-3. Mean days to germination of 'Dark Green Boston' lettuce seed matured under four day/night temperatures with a 12 hours photoperiod.

Seed maturation temp. (°C)	Germination temp. (°C) 27	30	33	36	Mean
	days				
20/10	4.6	5.3	6.7	7.0	5.9
25/15	2.8	3.1	5.4	5.1	4.1
30/20	1.6	2.2	2.7	2.1	2.2
35/25	1.3	2.1	2.9	3.2	2.4
Mean	2.6	3.2	4.4	4.4	
Contrasts (day temp.)					
20 + 25°C vs 30 + 35°C					**[z]
20°C vs 25°C					**
30°C vs 35°C					NS
Germination temp.					Q**
Seed maturation temp. x Germination temp.					NS

[z]NS,*,** Nonsignificant or significant at P=0.05 or 0.01, respectively, by F-test. Significant germination temp. effects were linear (L), quadratic (Q), or Cubic (C).

Table 3-4. Mean days to germination of 'Everglades' lettuce seed matured under four day/night temperatures with a 12 hours photoperiod.

	Germination temp. (°C)				
Seed maturation temp. (°C)	27	30	33	36	Mean
	days				
20/10	1.3	1.7	2.0	2.8	1.9
25/15	1.0	1.5	2.0	2.0	1.6
30/20	1.0	1.2	1.5	1.7	1.3
35/25	1.0	1.3	1.7	2.7	1.7
Mean	1.1	1.4	1.8	2.3	
Contrasts (Day temp.)					
20 + 25°C vs 30 + 35°C					*[z]
20°C vs 25°C					NS
30°C vs 35°C					NS
Germination temp.					L**
Seed maturation temp. x Germination temp.					NS

[z]NS,*,** Nonsignificant or significant at P=0.05 or 0.01, respectively, by F-test. Significant germination temp. effects were linear (L), quadratic (Q), or cubic (C).

Table 3-5. Mean days to germination of 'Valmaine' lettuce seed matured under four day/night temperatures with a 12 hours photoperiod.

	Germination temp. (°C)				
Seed maturation temp. (°C)	27	30	33	36	Mean
	days				
20/10	2.1	3.6	4.2	5.5	3.9
25/15	1.3	2.1	5.5	4.2	3.3
30/20	1.0	1.7	4.9	5.3	3.2
35/25	1.0	1.0	4.3	5.1	2.8
Mean	1.4	2.1	4.7	5.0	
Contrasts (Day temp.)					
20 + 25°C vs 30 + 35°C					NS[z]
20°C vs 25°C					NS
30°C vs 35°C					NS
Germination temp.					C**
Seed maturation temp. x Germination temp.					NS

[z]NS,*,** Nonsignificant or significant at P=0.05 or 0.01, respectively, by F-test. Significant germination temp. effects were linear (L), quadratic (Q), or cubic (C).

Table 3-6. Mean days to germination of 'Floricos 83' lettuce seed matured under four day/night temperatures with a 12 hours photoperiod.

Seed maturation temp. (°C)	Germination temp. (°C) 27	30	33	36	Mean
	days				
20/10	1.5	1.9	1.9	1.6	1.7
25/15	1.0	1.0	2.1	1.5	1.4
30/20	1.0	1.0	2.9	2.9	2.0
35/25	1.0	1.0	1.2	1.9	1.3
Mean	1.1	1.2	2.0	2.0	
Seed maturation temp. x Germination temp.					*[z]

[z]NS,*,** Nonsignificant or significant at P=0.05 or 0.01, respectively, by F-test.
$LSD_{(0.05)}$ value for seed maturation temp. x germination temp.=1.7.

Table 3-7. Mean days to germination of PI 251245 lettuce seed matured under four day/night temperatures with a 12 hours photoperiod.

	Germination temp. (°C)				
Seed maturation temp. (°C)	27	30	33	36	Mean
	days				
20/10	1.0	2.0	2.8	2.6	2.1
25/15	1.3	1.1	1.1	1.5	1.3
30/20	1.0	1.0	1.1	1.2	1.1
35/25	1.0	1.0	1.1	1.4	1.1
Mean	1.1	1.27	1.5	1.7	
Seed maturation temp. x Germination temp.					**[z]

[z]NS,*,** Nonsignificant or significant at P=0.05 or 0.01, respectively, by F-test.
$LSD_{(0.05)}$ value for seed maturation temp. x germination temp.=1.3.

'Dark Green Boston', a butterhead type, is grown during the winter months and is not adapted for production during periods when day temperatures exceed 25°C (Nagata et al., 1992). 'Everglades', also a butterhead type, has 'Dark Green Boston' in its genetic background. Guzman et al. (1992) reported that seeds of 'Everglades' have a thermotolerant character, so they will have in excess of 90% germination at temperatures up to 35°C.

'Valmaine' and 'Floricos 83' are cos types. 'Valmaine' is in the lineage of 'Floricos 83'. Even though 'Valmaine' is an outstanding commerical cultivar, it is susceptible to seed thermodormancy when soil temperatures rise above 30°C (Guzman, 1986; Guzman and Zitter, 1983). 'Floricos 83' can be successfully seeded early in the fall in south Florida since it germinates well in higher soil temperatures (Guzman and Zitter, 1983).

The thermotolerant character of 'Everglades' and 'Floricos 83' was proposed to be transfered from 'Maturo' (68288) (Guzman, 1986; Guzman et al., 1992; Guzman and Zitter, 1983). 'Maturo', a Spanish bibb type, was initially used as a source of resistance to lettuce mosaic virus (LMV) and has been attributed as the source of thermotolerance in all tolerant Florida cultivars (Guzman, 1986; Guzman et al., 1992; Guzman and Zitter, 1983). Therefore, it is possible that many LMV-resistant Florida genotypes may have thermotolerant genes in their background. To date, PI 251245, a wild lettuce accession, has been studied for its thermotolerant character. The PI line also has been used as parental material in breeding programs to confer resistance to lettuce mosaic virus (Nagata, 1993, pers. comm.). Seeds of this genotype had 100% germination at 30°C in this and other (Bradford, 1985) reseach; however, the expression of themotolerance was not consistent and was suggested to be dependent on where the seed was produced (Nagata, 1993, pers. comm.). Thermotolerant character could be inherited and this suggested the possibility of improving seed thermotolerance by breeding.

Germination of seed of some lettuce genotypes is controlled by light, which can also influence the upper temperature limit for seed germination (Evenari et al., 1953). In the

present research, all five genotypes germinated well at 24°C either in light or in darkness. At higher temperatures, light affected the response of thermotolerance on seed germination in all genotypes except 'Everglades'. Germination in darkness fell sharply when temperature increased above the upper optimum limits for germination. However, the addition of light during germination caused a rise of about 3°C in the upper temperature limit.

From the present results, seeds matured at high temperature had increased thermotolerance. Generally, the upper germination limit was increased when seeds were matured above 30°/20°C compared to the two lower temperature regimes. Koller (1962) reported that when 'Grand Rapids' lettuce seeds matured at 30°/23°C, the high-temperature tolerance for germination was 26°C in the light-break treatment. Gray et al. (1988b) reported that seeds of 'Saladin' produced at 30°/20°C germinated better at 30°C than those matured at 25°/15°C or 20/10°C temperature regimes. All of these observations demonstrated that, in certain lettuce genotypes, increasing the temperature at which seeds matured resulted in greater seed thermotolerance. Thermotolerance was regulated by interaction between genotype and maturation environment.

In general, seeds have an optimum germination temperature range which is sometimes very narrow. Even temperature increases of a few degrees (2° to 4°C) can decrease germination rate (Thompson, 1974). Seeds matured at 30°/20° or 35°/25°C germinated more rapidly and uniformly than those matured at lower temperatures; thusly, seed maturation temperature can also increase germination rate.

The suitable ambient temperature for increasing thermotolerance in lettuce seed was shown in the present results as well as in others. Koller (1962) indicated that good seed set in lettuce occurred at night temperatures between 17° and 23°C. In California, most lettuce seed is produced in the central San Joaquin Valley where daytime high temperatures may exceed 38°C for many consecutive days during lettuce seed production. Steiner and Opoku-Boateng (1991) reported that in Fresno, California, from August to September, 1988, the minimum temperatures during the day ranged from 11° to 22°C and the maximum

ranged from 30° to 40°C. This suggested that day/night temperatures of 30°/20°C may be an adequate natural range for induction of thermotolerance.

The underlying mechanism(s) of the effect of seed maturation temperature on embryogensis and seed development is unknown. In carrot, seeds matured at 30/20°C had higher DNA and rRNA content than those matured at 25°/15° or 20°/10°C and this finding was correlated with subsequent germination at high temperature (Gray et al., 1988b). In lettuce, the effect of temperature on DNA, RNA, or protein quality and quantity in seed is still unclear. If protein synthesis can be increased during the period of seed development and maturation at high temperature, the protein(s) may play a role in enhancing seed germination at high temperature. However, there is a lack of a clear and direct identification of the physiological and biochemical mechanism(s) by which maturing seed at high temperature enhances the thermotolerance in lettuce seed.

Lettuce seed size, weight, and total yield can be affected by seed maturation temperature (Drew and Brocklehurst, 1990; Steiner and Opoku-Boateng, 1991). When seeds in the present research were matured above 30°C, problems arose in both pollination and seed set, amount of seeds, and even the seed weight. However, as seed maturation temperature decreased, seed size and weight increased. Gray et al. (1988a) noted that in carrot an increase in temperature from 20°/10° to 30°/20°C reduced mean weight per seed by around 17%, but there was no effect of temperature on endosperm + embryo weight, or on endosperm cell number. In lettuce, the cell number per embryo + cotyledons of 'Saladin' seed was similar at 25°/15° and 30°/20°C, but both were lower compared with seeds grown at 20°/10°C (Gray et al., 1988b).

According to Fenner (1992) reduction in seed size at high production temperature could be due to the differential effect of temperature on the seed ripening and filling processes. The further increase in weight of seed matured at low temperature was due to reduced seed set and a longer period of seed filling, which allowed better use of photoassimilates and increased storage of carbohydrates. In the present experiment, plants

grown at 35°/25°C had pale green to yellowish leaves after two weeks; however, plants grown at 20°/10°C had green leaves for two months. The period for full seed maturation under lower temperature was 7 to 10 days longer than those matured under high temperature. It is possible that seeds matured at lower temperatures may be able to accumulate more nutrients and thus increase their size.

There is a positive correlation between seed size and seed vigor when seeds mature in the same environment. The larger or heavier the seed, the greater the germination percentage and the more vigorous the seedling in lettuce and other crops (Pollock and Roos, 1972; Soffer and Smith, 1974; Smith et al., 1973). The present results indicated that although seed size and weight were reduced when seed matured above 30°C; the seeds had greater thermotolerance. Therefore, the ability of the seed to germinate at high temperature was not related to an increase in seed weight.

Comparing seed yield, germination percentages, and the mean days to germination of the five genotypes under study, seeds matured at 30°/20°C had a greater germination percentage at high temperature than those matured at other temperatures. This means that the expression of thermotolerance in lettuce seed is regulated by a genotype-environment interaction.

Summary

Lettuce seed germination is strongly temperature dependent. Lettuce seeds differentially fail to germinate at temperatures above 24°C. This varies according to genotype. Varying thermotolerance traits are thought to be at least partly related to the environment under which the seed developed. In order to study the effects of temperature during seed development on the seed's subsequent germination, various lettuce genotypes were screened for their ability to germinate at temperatures from 20°C to 38°C. Seeds of

the selected genotypes 'Dark Green Boston' and 'Valmaine' (thermosensitive), 'Floricos 83', 'Everglades', and PI 251245 (thermotolerant) were produced at 20°/10°C, 25°/15°C, 30°/20°C, and 35°/25°C day/night temperature regimes in plant growth chambers. Seeds were germinated on a thermogradient bar from 24°C to 36°C under 12 hr light/dark cycles. As germination temperature increased, the number of seeds that failed to germinate increased. Above 27°C, seeds matured at 20°/10°C or 25°/15°C had a greater decrease in germination than did seeds matured at 30°/20°C and 35°/25°C. Seeds matured at temperatures of 30°/20°C or 35°/25°C had an increase in thermotolerance. Seeds of 'Dark Green Boston' and 'Everglades' matured at 30°/20°C had better thermotolerance than those matured at other temperatures. Germination of seeds of 'Valmaine' produced at 20°/10°C declined to 40% at 30°C, but seeds matured at other temperatures had over 95% germination. Above 30°C, however, germination of 'Valmaine' was not affected by seed maturation temperature. At 36°C, seeds of 'Floroicos 83' and PI 251245 had a greater germination (65% and near 100%, respectively) when produced at 35°/25°C than those produced at other temperatures. The effect of seed maturation temperature on the level of thermotolerance on germination varied according to genotype. Thermotolerance in lettuce seed appeared to be regulated by a interaction between genotype and environment. The upper temperature limit for germination of lettuce seed could be modified by manipulating the temperature during seed production. The potential thermtolerance of seed thereby increased, wherein thermosensitive genotypes became thermotolerant and thermotolerant genotypes (e.g., PI 251245) germinated fully at 36°C. This information is extremely useful for improveing lettuce seed germination during periods of high soil temperature, and can be used to study the biology of thermotolerance in lettuce.

CHAPTER 4
USING A PUNCTURE TEST TO IDENTIFY THE ROLE OF SEED COVERINGS ON GERMINATION OF THERMOTOLERANT LETTUCE SEED

Introduction

Botanically, the seed of lettuce (*Lactuca sativa* L.) is an achene. Seed coverings that surround the embryo include the pericarp, integument, and endosperm (Borthwick and Robbins, 1928). For germination to occur, the embryonic axis must penetrate these layers. In certain lettuce genotypes, when the temperature is above 30°C, seed germination is erratic or completely inhibited, a condition termed thermoinhibition (Gray, 1975; Khan, 1980/81). At high temperature, germination of lettuce seed is problematic because the seed coverings can act as a physical barrier restricting germination (Ikuma and Thimann, 1963; Speer, 1974). Cutting or removing the endosperm, integument and pericarp from lettuce seed prevents thermoinhibition (Ikuma and Thimann, 1963b). Weakening of the tissues opposite the radicle tip can be sufficient to remove this constraint, permitting the radicle to emerge.

Seed priming is a technique that can improve germination and make it more uniform. Priming is a controlled hydration process that permits pregerminative metabolic activity to proceed but prevents radicle emergence. According to Khan (1980), seed priming initiates several physiological and biochemical changes in seeds, so it can shorten the period needed for seed germination and bypass the restrictions to germination at supraoptimal temperatures.

The uptake of water by seeds is an essential initial step toward germination. When dry viable seeds imbibe water, a chain of events is initiated that ultimately results in the emergence of the radicle. Under optimal conditions, the uptake of water by seed

is triphasic (Bewley and Black, 1994). In phase I, the water potential of a mature dry seed is much lower than that of the surrounding moist substrate, and water uptake increases sharply. The Q_{10} value of imbibition during phase I is 1.5-1.8, indicating that imbibition is a physical process not dependent on metabolic energy and is related to the properties of colloids, such as proteins, present in seed tissue.

During phase II, the lag phase, water uptake slows or stops. The seed undergoes many processes essential for germination. Phase II is primarily a time of chemical activation and its length can be affected by many factors, including temperature, water deficit, or irradiation (Bradford, 1990). Bradford indicated that the length of phase II was determined by the time required to reduce the threshold of penetration the seed coverings. Georghiou et al. (1983) observed cracks and breaks between the pericarp, integument, and endosperm of lettuce seeds just prior to visible radicle growth, leading to a reduction in the threshold for penetration of the seed coverings.

After the radicle protruded, the water content could increase again (phase III). The initiation of radicle growth is influenced by tissues surrounding the embryo in such crops as tomato (Groot and Karssen, 1987), pepper (Watkins and Cantliffe, 1983), celery (Jacobsen et al., 1976), muskmelon (Welbaum et al., 1995), and lettuce (Nabors and Lang, 1971). For germination to occur, the embryonic axis must penetrate endosperm or perisperm, integument, and pericarp. To achieve this, the hydrostatic pressure of the embryo must be increased. This is accomplished in seeds by the accumulation of osmotic solutes (Takeba, 1980a) that allow the expanding radicle to rupture the barrier tissue when its hydrostatic pressure exceedes the threshold necessary for penetration (Nabors and Lang, 1971a; Takeba, 1980a). In tomato and lettuce, however, direct measurements of solute potential in excised axes have failed to account completely for the increases in growth potential observed (Haigh and Barlow, 1987; Nabors and Lang, 1971b). This indicates that decreased minimum resistance to

penetrate the endosperm or increased cell wall extensibility of the endosperm cell may be responsible for radicle growth during germination (Carpita et al., 1979).

Cell-wall-degrading enzymes located in the micropylar region of the endosperm tissue may be active and may be a necessary prerequisite for normal germination (Ikuma and Thimann, 1963b). During germination, weakening of the endosperm tissue around the radicle just prior to radicle protrusion has been noted in pepper and tomato (Groot and Karssen, 1987; Watkins and Cantliffe, 1983) and this may apply also to lettuce. Mannanase, cellulase, pectinase, and pentosanase can be effective in promoting germination of dormant lettuce seed, although it has not been established that these enzymes mediate lettuce endosperm wall weakening *in vivo* prior to radicle emergence (Bewley and Halmer, 1980/81; Halmer et al., 1976; Ikuma and Thimann, 1963b).

To analyze the role of the seed coverings in seed germination of pepper, tomato, muskmelon and lettuce, puncture tests by Tao and Khan (1979), Watkins and Cantliffe (1983), Groot and Karssen (1987), and Welbaum et al. (1995) were conducted to quantify the force required to penetrate the covering tissues. At radicle emergence in tomato seed, the force required to puncture endosperm tissue was 0.2 newtons (N) (Groot and Karssen, 1987), and in pepper seed it was 0.3N (Watkins and Cantliffe, 1983). In a similar test, the net force required to puncture the perisperm envelope of muskmelon at radicle emergence was 0.1N (Welbaum et al., 1995). In lettuce seed, the force required to penetrate endosperm of 'Grand Rapids' seed was 0.6N (Tao and Khan, 1979). Drew and Blocklehurst (1984) indicated that the value of the strength of the endosperm was 0.5-0.6N in 'Cobham Green' but they could not detect differences in endosperm penetration forces that were consistent with high-temperature germination behavior. Wurr et al. (1987) reported that in 'Ithaca', 'Pennlake', and 'Saladin' lettuce, a force of 0.3-0.4N was required to penetrate the endosperm and 1.5N-1.7N to penetrate the whole seed. These results suggested that the strength of the pericarp rather than

the strength of the endosperm may be the major determinant of germination at high temperature in the examined cultivars.

To determine the role of the seed coverings in restricting lettuce seed germination at high temperature, we employed puncture tests to measure the force required to penetrate the various parts of the seed coverings. Seed of two thermosensitive and three thermotolerant genotypes were used. Priming was conducted in all cases in order to compare puncture force among seeds where thermodormancy was known to be completely bypassed.

Materials and Methods

Five lettuce genotypes varying in their degree of thermotolerance were used in this study: 'Dark Green Boston' and 'Valmaine' (thermosensitive), and 'Floricos 83', 'Everglades', and PI 251245 (thermotolerant). All seeds were produced in the Salinas area of California in 1992.

Priming Treatment

Polyethylene glycol (PEG) was used as osmoticum. The osmotic potentials of the PEG solutions were -1.0, -1.1, -1.2, and -1.3 Mpa (Michel and Kaufmann, 1973). After testing the combination series of priming situations for each genotype, i.e., concentrations of PEG for various durations, the best conditions for priming seed of the five genotypes were listed in Table A-5.

Seeds were weighed then placed in 200 mm test tubes with 30 ml of soak solution per g of seed. Test tubes were covered with 4 x 4 cm^2 pieces of Parafilm and placed in an incubator at 15°C with constant light (~10 μmol m^{-2} s^{-1}). Appropriate aeration during priming was provided by means of a glass tube connected by a rubber hose to an aquarium pump. After priming for 2 to 6 days, the seeds were placed in a

Buchner funnel, then rinsed with 100 ml of distilled water. Surface moisture was removed by 1 min of suction. The rinse process was performed twice. Seeds were placed in 9 cm Petri dishes and set in an incubator at 10°C and 45% RH for at least 1 day until the weight of the seeds returned to the pretreatment weight. Seeds were stored under these conditions until used.

Germination Tests

Germination tests were conducted at 36°C in 12 hour light (~20μmol $m^{-2} s^{-1}$) -12 hour dark periods. Thirty seeds per replication per treatment were placed in a 5.5 cm Petri dish with two layers of 4.5 cm diameter Whitman #3 filter paper moistened with 1-2 ml of distilled water. Distilled water was added as needed to keep the filter paper moist. Germination was defined as visible radicle protrusion through the pericarp, and total percent germination was calculated. A complete randomized experimental design with genotypes and priming treatment was replicated three times.

Moisture Content

Primed and nonprimed seeds were imbibed in an incubator at 36°C for various lengths of time under 12/12 hour light/dark periods. Primed seeds were weighed hourly during imbibition until the radicle emerged. Because of poor or no germination in nonprimed seeds of 'Dark Green Boston', 'Valmaine', and 'Floricos 83' at 36°C, water content of seeds of the three genotypes were measured at the same hour as those of primed seeds. To measure dry weight, the seeds were oven dried for 1 hour at 130°C, then were cooled in a desiccator for 20 minutes, following the Rules of the International Seed Testing Association (1985). Seed moisture content as a percentage of weight was calculated using the following formula:

$$(\text{fresh weight} - \text{dry weight}) / \text{fresh weight} \times 100.$$

All treatments were replicated three times.

The Puncture Test

Primed and nonprimed seeds of the five genotypes were imbibed by the same method used for the germination test. Eighty seeds of each genotype were imbibed for six hours at 24°C and 33°C. An additional 80 seeds of each genotype at each duration were imbibed at 36°C as follows: 'Valmaine', 'Floricos 83', and 'Everglades', all for 1, 3, and 5 hours; PI 251245 for 1, 2, and 3 hours; and 'Dark Green Boston' for 1, 3, 5, and 9 hours. The last hour at which each genotype was measured was correlated to the hour before radicle protrusion. The hours in between always included the first hour after imbibition and the middle hour between first and last. Before the puncture test, imbibed seeds were cut 1mm above the radicle tip under a stero microscope (Olympus SZ 30). Cut seeds were left with the seed coat intact (Achene), had the pericarp and integument removed from the achene (Ed+Em), or were left with the embryo only (Em) (Figure 4-1).

The puncture test was conducted using an Instron Model 1132 Universal Testing Machine (Instron Corporation, Canton, MA). The load cell was set to 200g full scale load. Crosshead and chart speed were 5 and 10 cm/min, respectively. The seed tissue was placed in a countersink on an aluminum block (4 x 4 cm^2) directly above a hole drilled through the block (Figure 4-2). A circular flat-faced No. 92 drill bit, 0.20 mm in diameter, was attached to the load cell of the machine. As the drill bit was lowered, it penetrated the cotyledons, embryonic axis, endosperm, integument, and pericarp, which finished one measurment. This process was monitored under a 2X magnifier with light.

The force required to penetrate the seed tissues was recorded and was determined from the peak of the load-deflection curve (Figure A-1). During the test, the load lowered and the drill passed through the cotyledon, embryonic axis, endosperm, embryo, integument, and pericarp; when following this process there was a slight

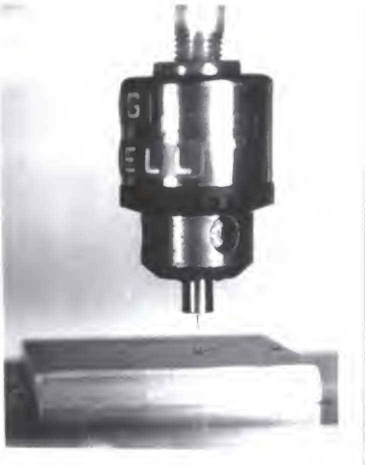


Figure 4-1. The lettuce seed section attached to the drill in the puncture test with the Instron Testing Machine.

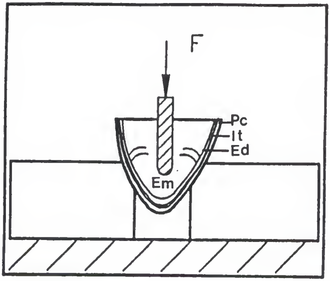


Figure 4-2. The drill penetrated the achene pieces which were placed in a aluminum block.
Pc: pericarp; It: integument; Ed: Endosperm; Em: embryo.

change in slope of the load-deformation curve, after the drill penetrated through the pericarp when the curve finally plateaued. The force required to penetrate the seed tissues was measured in newton units (N) which is the SI standard unit of force; one newton of force equals the force generated by gravity on 101.97g (Bourne, 1982).

To be sure the drill passed through the tip of the seed, the procedure for each treatment was repeated until data of 5 to 7 similar punctures were collected. All treatments were replicated three times.

Experimental Design and Statistical Analysis

The puncture tests were conducted using a randomized complete block design, with each treatment replicated three times. A Statistical Analysis System (SAS) software program (SAS, 1987) was used to analyze the data. The main effects were seed priming and imbibition hours. In analyzing the effect of priming, treatment means were separated by the Least Significant Difference (LSD) test.

Results

Seed Priming Treatment

Germination of primed and nonprimed seeds of the five genotypes at 36°C is listed in Table A-6. Although germination at 36°C was completely inhibited in the two nonthermotolerant genotypes, 'Dark Green Boston' and 'Valmaine', priming almost completely circumvented this inhibition. When primed, all thermotolerant genotypes germinated 100% at 36°C. Two of those germinated about 93% without priming.

Water Content

Among the five genotypes, seed water content increased with time and had the same triphasic pattern for primed seeds and nonprimed seeds (Figures 4-3 to 4-7). Seed water content quickly increased during the first hour of imbibition, then reached a plateau, and then increased after radicle protrusion.

In 'Dark Green Boston', the water content of both primed and nonprimed seeds was 45%, and primed seeds germinated after 11 hours imbibition (Figure 4-3). Nonprimed seeds did not germinate. In 'Valmaine', water content of primed seeds was 5% more than that of nonprimed seeds after one hour imbibition; primed seeds germinated after soaking in water for seven hours (Figure 4-4). After one hour of imbibition, water content of primed seeds of 'Floricos 83', 'Everglades', and PI 251245 was 5% more than that of nonprimed seeds, and continued to increase gradually for the next four to five hours, at which point the radicle emerged (Figures 4-5 to 4-7, respectively). Water content of nonprimed seeds of 'Floricos 83', 'Everglades', and PI 251245 lagged behind that of primed seed, then increased 7% to 10% at three, six, and four hours of imbibition, respectively, after which water content remained unchanged. Nonprimed seeds of 'Floricos 83', 'Everglades', and PI 251245 germinated at 36°C, but germination of 'Floricos 83' was lower, so its water content was measured for eight hours of imbibition. Radicles of nonprimed 'Everglades' and PI 251245 seed protruded through the seed coverings after 10 hours imbibition and primed seeds germinated faster than nonprimed seeds.

Puncture Test

When seeds of all genotypes were imbibed at 24° or 33°C for 6 hours, there was no significant interaction between genotype and imbibition temperature in the force required to penetrate the achene and the endosperm (Table 4-1). However, the force

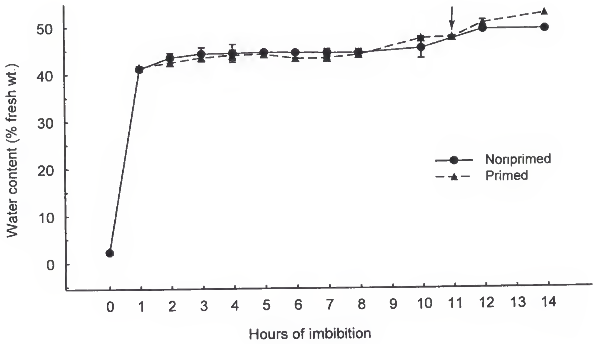


Fig 4-3. Water content of 'Dark Green Boston' lettuce seeds during imbibition at 36 C. Arrow indicates time of radicle protrusion. Vertical bars indicate standard error.

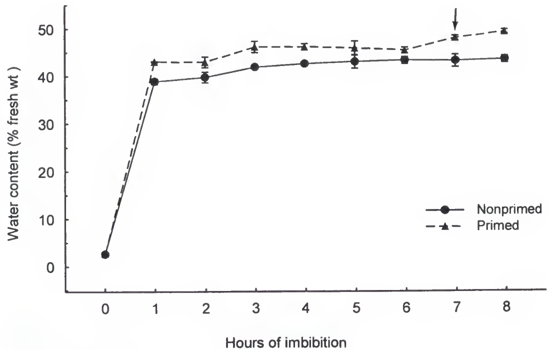


Fig 4-4. Water content of 'Valmaine' lettuce seeds during imbibition at 36 C. Arrow indicates time of radicle protrusion. Vertical bars indicate standard error.

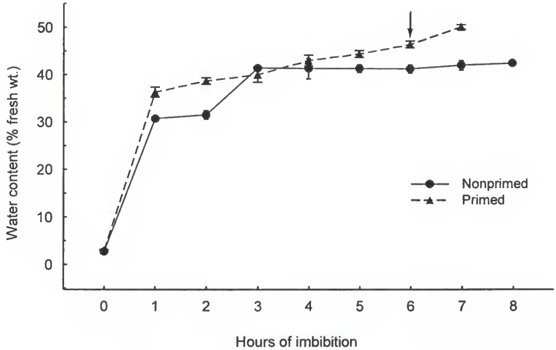


Fig 4-5. Water content of 'Floricos 83' lettuce seeds during imbibition at 36 C. Arrow indicates time of radicle protrusion. Vertical bars indicate standard error.

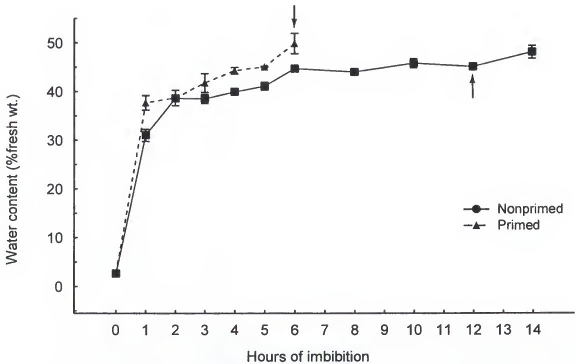


Fig 4-6. Water content of 'Everglades' lettuce seeds during imbibition at 36 C. Arrows indicate time of radicle protrusion. Vertical bars indicate standard error.

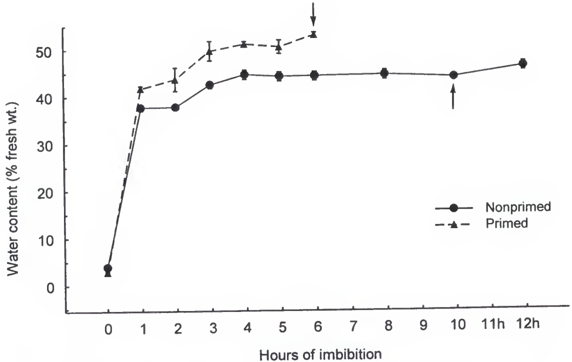


Fig 4-7. Water content of PI 251245 lettuce seeds during imbibition at 36 C. Arrows indicate time of radicle protrusion. Vertical bars indicate standard error.

Table 4-1. Mean force required to penetrate lettuce seed tissues after 6 hours of imbibition at 24°C and 33°C.

Genotype	Force (Newton) 24°C	Force (Newton) 33°C	Mean
	Achene		
Dark Green Boston	0.208	0.206	0.207
Valmaine	0.178	0.202	0.190
Floricos 83	0.214	0.148	0.181
Everglades	0.180	0.144	0.162
PI 251245	0.128	0.150	0.139
Mean	0.182	0.170	
Genotype (LSD $_{(0.05)}$=0.043)[y]			*[z]
Temp.			NS
Genotype x Temp.			NS
	Ed+Em		Mean
Dark Green Boston	0.080	0.104	0.092
Valmaine	0.097	0.118	0.108
Floricos 83	0.114	0.093	0.103
Everglades	0.077	0.088	0.083
PI 251245	0.099	0.092	0.096
Mean	0.093	0.099	
Genotype			*
Temp.			NS
Genotype x Temp.			*
Genotype x Temp. LSD $_{(0.05)}$=0.02			
	Em		Mean
Dark Green Boston	0.072	0.072	0.070
Valmaine	0.083	0.088	0.086
Floricos 83	0.073	0.079	0.076
Everglades	0.065	0.071	0.068
PI 251245	0.077	0.080	0.079
Mean	0.074	0.078	
Genotypes (LSD $_{(0.05)}$=0.010)			*
Temp.			NS
Genotype x Temp.			NS

[z]NS, *,** Nonsignificant or significant at P=0.05, or 0.01, respectively, by F-test.
[y] Mean separation within treatments by Least Significant Difference test at P=0.05.

required to penetrate the achene alone was different among the five genotypes. 'Dark Green Boston' had the greatest average resistance (0.207N) and PI 251245 the least (0.139N)

When the temperature during imbibition was increased, the force required to penetrate the endosperm in the five genotypes was different (Table 4-1). For example, at 24°C, the force required for 'Valmaine' and 'Floricos 83' was 0.097N and 0.114N, respectively. At 33°C, the force required for 'Valmaine' increased to 0.118 N, but in 'Floricos 83' decreased to 0.093N. In 'Dark Green Boston' and 'Everglades', the forces were similar and both forces increased at 33°C compared to those at 24°C, but the increased force in 'Dark Green Boston' was more than that in 'Everglades'. In the PI line, this force significantly decreased at 33°C. The forces required to penetrate the embryo were measured and recorded as reference data only and will not be discussed.

When primed and nonprimed seeds of the five genotypes were imbibed at 36°C for various hours, there was no significant difference in the effect of the interaction between priming and imbibition hours. In 'Dark Green Boston', the force required to penetrate nonprimed achenes was significantly greater than for primed achenes, even after nine hours of imbibition (Table 4-2). For primed seed, the required force declined only from 0.169 to 0.163 between the first hour and after nine hours of imbibition; for nonprimed seed, the force declined after three hours. The force required to penetrate the endosperm in primed seed was decreased by imbibition time; in nonprimed seeds, it increased after five hours of imbibition.

In 'Valmaine', seed priming did not affect the force necessary to penetrate the endosperm (Table 4-3). The penetration force of the achene was affected by imbibition time and sharply declined after two hours of imbibition.

In 'Floricos 83', priming had a significant effect on the force necessary to penetrate the achene (Table 4-4). The force required to penetrate a nonprimed achene (0.183N) was greater than that for primed achene (0.163N). During the first two hours

Table 4-2. Mean force required to penetrate 'Dark Green Boston' lettuce seed tissues during imbibition at 36°C.

Hours	Force (Newton)		
	Primed	Nonprimed	Mean
	Achene		
1	0.169	0.207	0.188
3	0.170	0.204	0.187
5	0.166	0.192	0.179
9	0.163	0.186	0.175
Mean	0.167	0.197	
Prime (LSD $_{(0.05)}$= 0.011)[y]			**[z]
Hour			NS
Prime x Hour			NS
	ED+Em		Mean
1	0.115	0.093	· 0.014
3	0.112	0.094	0.103
5	0.108	0.094	0.101
9	0.103	0.109	0.106
Mean	0.109	0.097	
Prime (LSD $_{(0\ 05)}$= 0.009)			*
Hour			NS
Prime x Hour			NS
	Em		Mean
1	0.071	0.083	0.077
3	0.090	0.084	0.087
5	0.092	0.086	0.089
9	0.093	0.092	0.093
Mean	0.086	0.086	
Prime			NS
Temp (LSD $_{(0\ 05)}$= 0.009)			*
Prime x Hour			NS

[z]NS, *,** Nonsignificant or significant at P=0.05, or 0.01, respectively, by F-test.
[y]Mean separation within treatments by Least Significant Difference test at P=0.05.

Table 4-3. Mean force required to penetrate 'Valmaine' lettuce seed tissues during imbibition at 36°C.

Hours	Force (Newton) Primed	Force (Newton) Nonprimed	Mean
	Achene		
1	0.176	0.183	0.179
2	0.164	0.160	0.162
3	0.129	0.150	0.139
5	0.127	0.139	0.133
Mean	0.149	0.158	
Prime			NS[z]
Hour (LSD $_{(0.05)}$=0.030)[y]			**
Prime x Hour			NS
	Ed+Em		Mean
1	0.106	0.106	0.106
2	0.105	0.103	0.104
3	0.104	0.102	0.103
5	0.102	0.101	0.105
Mean	0.104	0.103	
Prime			NS
Hour			NS
Prime x Hour			NS
	Em		Mean
1	0.094	0.081	0.087
2	0.093	0.085	0.089
3	0.095	0.091	0.093
5	0.097	0.091	0.094
Mean	0.095	0.087	
Prime			NS
Hour (LSD $_{(0.05)}$=0.005)			*
Prime x Hour			NS

[z]NS, *, ** Nonsignificant or significant at P=0.05 or 0.01, respectively, by F-test.
[y]Mean separation within treatments by Least Significant Difference test at P=0.05

Table 4-4 . Mean force required to penetrate 'Floricos 83' lettuce seed tissues during imbibition at 36°C.

Hours	Force (Newton)		
	Primed	Nonprimed	
	Achene		Mean
1	0.186	0.189	0.188
2	0.181	0.189	0.185
3	0.147	0.186	0.167
5	0.139	0.169	0.154
Mean	0.163	0.183	
Prime ($LSD_{(0.05)}$=0.014)[y]			*[z]
Hour ($LSD_{(0.05)}$=0.016)			*
Prime x Hour			NS
	Ed+Em		Mean
1	0.122	0.116	0.119
2	0.112	0.117	0.115
3	0.107	0.105	0.106
5	0.104	0.106	0.105
Mean	0.111	0.111	
Prime			NS
Hour			NS
Prime x Hour			NS
	Em		Mean
1	0.118	0.095	0.107
2	0.110	0.094	0.102
3	0.104	0.090	0.097
5	0.097	0.094	0.096
Mean	0.107	0.093	
Prime ($LSD_{(0.05)}$=0.011)			*
Hour			NS
Prime x Hour			NS

[z]NS, *,** Nonsignificant or significant at P=0.05, or 0.01, respectively, by F-test.
[y]Mean separation within treatments by Least Significant Difference test at P=0.05

of imbibition, penetration forces in primed and nonprimed seeds were similar. After three hours of imbibition, the penetration force in the primed achene abruptly dropped, although there was no reduction in nonprimed seed until the fifth hour of imbibition. For the endosperm, there was no significant differences in the penetration forces of primed and nonprimed seed.

In 'Everglades', priming had a significant effect on the force necessary to penetrate both the achene and the endosperm (Table 4-5). Force required to pentrate the endosperm in nonprimed seeds had an average of 0.01N greater resistance than that for primed seeds. The force required to penetrate the achene in primed seeds was lower than that in nonprimed seeds. As imbibition time increased, penetration force significantly decreased.

The puncture force for the achene and endosperm of PI 251245 was the same for both nonprimed and primed seeds (Table 4-6).

For a better understanding of the effect of priming on puncture force, achene and endosperm data were taken after the first hour of imbibition and the hour before radicle protrusion (Figures 4-8 and 4-9). The force required to penetrate the achene was affected by genotype, seed priming, and imbibition time. Except for 'Dark Green Boston', puncture force decreased in both primed and nonprimed seed in all genotypes during the period of imbibition studied. The force required to penetrate the achene in primed seeds of 'Dark Green Boston' was lower than that required in nonprimed seed after one hour of imbibiton, and this puncture force remained unchanged after nine hours of imbibition. The force required to penetrate the endosperm in nonprimed seed of 'Dark Green Boston' increased after nine hours of imbibition. This puncture force was less in primed seeds. The force required to penetrate the achene of 'Valmaine' decreased with imbibed time, especially in primed seed. The force necessary to penetrate the endosperm was the same for both primed and nonprimed seeds of 'Valmaine'. In the thermotolerant genotypes 'Floricos 83', 'Everglades', and PI 251245,

Table 4-5. Mean force required to penetrate 'Everglades' lettuce seed tissues during imbibition at 36°C.

Hours	Force (Newton)		
	Primed	Nonprimed	
	Achene		Mean
1	0.156	0.218	0.187
2	0.131	0.171	0.151
3	0.112	0.148	0.130
5	0.099	0.121	0.110
Mean	0.125	0.165	
Prime (LSD $_{(0.05)}$=0.016)[y]			**[z]
Hour (LSD $_{(0.05)}$=0.031)			**
Prime x Hour			NS
	Ed+Em		Mean
1	0.091	0.113	0.102
2	0.085	0.096	0.091
3	0.086	0.093	0.090
5	0.085	0.087	0.086
Mean	0.087	0.097	
Prime (LSD $_{(0.05)}$=0.008)			*
Hour			NS
Prime x Hour			NS
	Em		Mean
1	0.074	0.071	0.073
2	0.085	0.078	0.082
3	0.085	0.080	0.083
5	0.079	0.080	0.080
Mean	0.081	0.077	
Prime			NS
Hour			NS
Prime x Hour			NS

[z]NS, *,** Nonsignificant or significant at P=0.05, or 0.01, respectively, by F-test.
[y]Mean separation within treatments by Least Significant Difference test at P=0.05

Table 4-6. Mean force required to penetrate 'PI251245' lettuce seed tissues during imbibition at 36°C.

Hours	Force (Newton)		
	Primed	Nonprimed	
	Achene		Mean
1	0.138	0.150	0.144
2	0.127	0.147	0.137
3	0.105	0.119	0.112
Mean	0.123	0.138	
Prime			NS[z]
Hour			NS
Prime x Hour			NS
	Ed+Em		Mean
1	0.106	0.109	0.108
2	0.102	0.113	0.108
3	0.095	0.104	0.100
Mean	0.101	0.109	
Prime			NS
Hour			NS
Prime x Hour			NS
	Em		Mean
1	0.097	0.094	0.096
2	0.097	0.091	0.094
3	0.094	0.092	0.093
Mean	0.096	0.092	
Prime			NS
Hour			NS
Prime x Hour			NS

[z]NS, *, ** Nonsignificant or significant at P=0.05 or 0.01, respectively, by F-test.

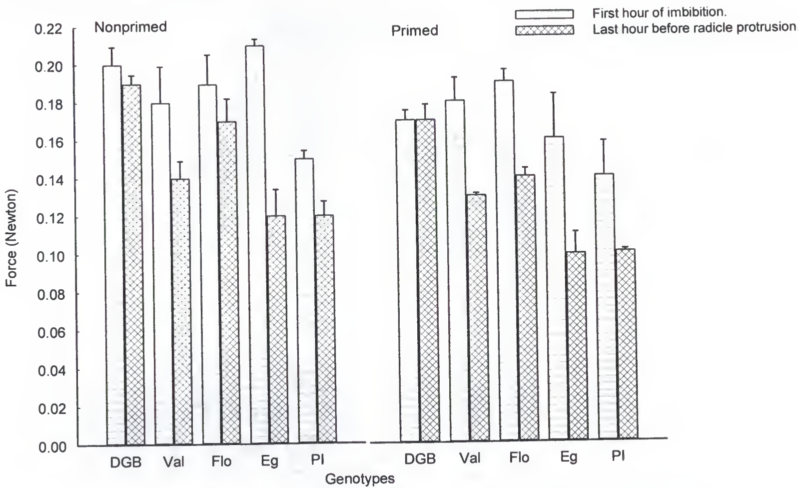


Figure 4-8. Force required to penetrate lettuce achene at 36C with two various hours of imbibition. DGB: Dark Green Boston; Val: Valmaine; Flo: Floricos 83; Eg: Everglades; PI: PI 251245. Vertical bars indicate standard error.

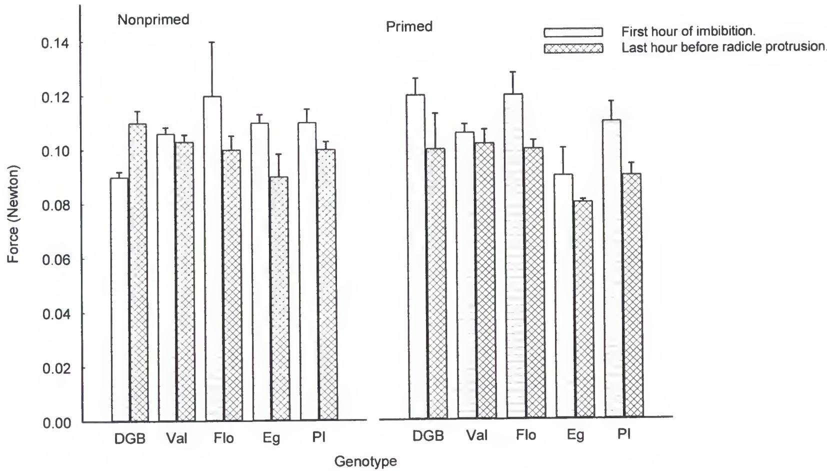


Figure 4-9. Force required to penetrate lettuce endosperm at 36C with two various hours of imbibition.
DGB: Dark Green boston; Val: Valmaine; Flo: Floricos 83; Eg: Everglades; PI: PI 251245.
Vertical bars indicate standard error.

penetration forces for the achene and the endosperm decreased during imbibition approaching radicle emergence for both primed and nonprimed seeds.

Discussion

To identify the role of the seed coverings in controlling seed germination at high temperature, Tao and Khan (1979), Drew and Brocklehurst (1984 and 1990), and Wurr et al. (1987) used a puncture test to measure the force required to penetrate the different lettuce seed tissues. In 'Grand Rapids' and 'Cobham Green', the major barrier to embryo growth was reported to be the endosperm layers, which contributed 60% and 40%, respectively, of the total resistance needed to puncture the intact seed (Drew and Brocklehurst, 1984; Tao and Khan, 1979). However, Wurr et al. (1987) suggested that the strength of the pericarp played a more important role in determining germination than did that of the endosperm.

The critical role of the endosperm or the pericarp on resistance to lettuce seed germination at high temperature still was not clear from these puncture tests. Possibly this was due to the fact that different cultivars of lettuce were used or that the position used to penetrate the seeds varied from researcher to researcher. To measure the force required to penetrate the seed coverings, we determined that the puncture area must be where the radicle would naturally protrude through the seed coverings. If this was properly done, the measurement of seed-layer strength may be more correlated to restriction of embryo growth. The work of Drew and Brocklehurst (1984) and Wurr et al. (1987) used a 0.7mm probe and penetrated the center of the whole-seed specimens dorso-ventrally. In our puncture tests, we used a 0.2mm probe and penetrated through the micropyle to imitate the route of the radicle, since it has been reported that only the micropylar endosperm cells undergo significant structural alterations before radicle emergence in germinating lettuce seeds (Georghiou et al., 1983; Psaras et al., 1981).

In this work, we attempted to measure the precise force required to penetrate the seed coverings. This method of measurement presented some problems since identifying the exact site to take measurements was difficult. In addition, it was necessary to repeat the procedure several times to get the accurate data, thereby increasing the chances of freedom of movement of the machine.

To determine if this restraint was related to genotypic thermotolerance, the five lettuce genotypes used in the present research had different abilities to germinate at high temperatures. At imbibition temperatures of 24°C and 33°C, less forces was required to penetrate the achene or endosperm or both of seeds of three thermotolerant genotypes than those of two thermosensitive genotypes. These penetration forces increased or remained unchanged, however, in the two thermosensitive genotypes. These differences suggested that the resistance of the seed coverings could be decreased at high germination temperature in the thermotolerant genotypes.

During phase II of the imbibition process, water uptake by seed slows or stops, and the seed undergoes many processes essential for germination. In this phase, water content in lettuce seed was around 40%, compared to the 3-4% present in dry seed. Phase II is primarily a period of chemical activation and its length can be affected by several factors, including temperature, water deficit, or irradiation (Bradford, 1990). Primed seeds appeared to have a greater water content and a shorter lag phase than nonprimed seeds. This may be due to the activation of hydratable substances and an improvement in seed coat permeability. Perhaps the tissues surrounding the embryo offered less resistance after priming, which enhanced their ability to take up more water. In addition, it may be that the radicle in primed seeds was already in a cell division and cell elongation phase (Bradford, 1986; Heydecker et al., 1975).

Bradford (1990) indicated that the length of phase II was determined by the time required to reduce the resistance of the seed coverings, although at the time of radicle emergence the barrier formed by the seed coverings constitutes only a minimal

resistance to radicle growth. Additionally, the turgor pressure of the embryo may be sufficient enough for the radicle to overcome the remaining resistance of the seed coverings. Seed coverings also help to establish a water potential gradient to initiate phase III of imbibition. Georghiou et al. (1983) observed cracks and breaks between the pericarp, integument, and endosperm just prior to visible radicle growth, leading to a reduction in the yield threshold of penetration of the seed coverings (minimum embryo turgor required for penetration of seed coverings). Since intact seeds are at zero water potential during phase II of imbibition, no further water uptake can occur at that time. At the transition between phase II and phase III, when the seed coverings rupture due to the combined effects of embryo turgor and tissue weakening, tension on the embryo is released and the radicle can protrude, signifying that germination has been successfully completed (Welbaum and Bradford, 1990). It is at this point, at phase III when the radicle protrudes through the seed coverings, that the water content of seeds increases again due to continued cell division and subelongation. In the present research, nonprimed seeds did not germinate uniformly and this rapid increase in water uptake at phase III was not detected.

The puncture forces for achene and endosperm of primed and nonprimed seeds of the three thermotolerant genotypes decreased during imbibition at 36°C. Priming appeared to lower the initial force required to penetrate the achene and the endosperm, especially in 'Everglades'. In primed seed, measurement of puncture force was stopped just before radicle protrusion. At this time, nonprimed seeds of 'Floricos 83' and 'Valmaine' did not have as great a reduction in puncture force as primed seeds. It might be possible that the amount of reduction was not sufficient to allow penetration of the achene, which may explain why these seeds did not germinate well at 36°C. Primed 'Dark Green Boston' seeds required less force to penetrate the endosperm than nonprimed seeds, while the puncture force of nonprimed seeds increased after nine hours of imbibition. It appeared that priming lead to a lowering of the initial force

necessary to penetrate the seed coat and that this reduction was enough to overcome the resistance of seed coverings when germination took place at 36°C.

These results suggested that for seeds to germinate at high temperature, first there must be a decrease in the resistance of the endosperm layers and, second, the pericarp and integument must be weakened enough so that the resistance is less than the tugor pressure of the embryo. Bradford (1990) suggested that the yield threshold (minimum turgor of growth) for germination is determined by the yield threshold of tissues surrounding the embryo (minimum embryo turgor required for penetration the seed coverings) and the radicle itself (minimum turgor for growth of the embryonic axis). For example, Tao and Khan (1979) reported that the force required to penetrate the endosperm of 'Grand Rapids' lettuce seed treated with gibberellic acid was 0.15N lower than that of nontreated seeds. In pepper seed, application of gibberellic acid resulted in earlier germination at low temperature and a decrease in endosperm strength earlier than in untreated seeds (Watkins and Cantliffe, 1983). Gibberellic acid increased galactomannanase activity during germination before radicle emergence in pepper. This indicated that, before radicle emergence, there occurred a breakdown and loss of cellular integrity of the endosperm and a subsequent reduction in endosperm thickness directly in front of the radicle (Watkins et al., 1985). Germination studies with gibberellin-deficient mutant tomato seeds proved that endosperm weakening absolutely depended on gibberellin since it was shown to induce enzymatic hydrolysis of the relatively thick cell walls of the endosperm. These enzymes included endo-β-mannanase, α-galactosidase, and mannohydrolase (Groot et al., 1988).

It has been suggested that, for lettuce seed, the emerging radicle can develop enough thrust to overcome the estimated mechanical resistance of the endosperm without first degrading the endosperm cell wall (Nabors and Lang, 1971a). In the present study, the turgor of the embryonic axis was not measured; however, it did not appear that the embryo forces constituted a considerable barrier (Bradford,1990).

Rather, the results of this study suggest that it was the endosperm that presented the resistance when radicle growth began. This force may have been reduced as a result of priming via preliminary weakening that reduced the penetration resistance of the seed coverings of thermotolerant genotypes. Thermotolerant genotypes and primed seeds required less penetration force and may either have had a weakened integument and pericarp or a weakened endosperm. Present results indicated that the timing of radicle emergence was controlled primarily by the rate at which weakening of the seed coverings occurred.

Summary

Temperature is an important environmental factor that affects lettuce germination. At high temperature, the seed coverings can act as a physical barrier, restricting germination. Weakening of the tissues in front of the radicle tip can be sufficient to permit the radicle to emerge. The present research was conducted to determine the role of seed coverings on seed germination at high temperature. Two thermosensitive and three thermotolerant lettuce genotypes were primed in order to bypass thermoinhibition. During germination of primed and nonprimed seeds, imbibition followed a normal triphasic pattern. Primed seeds had a higher water content, a shorter imbibitional phase II, and germinated at 36°C, as compared to nonprimed seeds where the thermosensitive genotypes did not germinate at high temperature. These differences indicated that physiological and biochemical changes had occurred in primed seeds. These changes may have shortened the period needed for seed germination, thus bypassing the restrictions to germination at supraoptimal temperatures.

Puncture tests were conducted to measure the force required to penetrate the whole seed (achene) and endosperm of the five genotypes. Genotype, not temperature,

determined the forces necessary to penetrate the achene in seeds imbibed for six hours at 24°C and 33°C,. 'Dark Green Boston', a thermosensitive genotype, had the greatest average resistance (0.207N) and PI 251245, a thermotolerant genotype, had the least (0.139N). Changes of forces required to penetrate the endopserm in the five genotypes for both temperatures were different. However, the three thermotolerant genotypes had lower endosperm resistance than the two thermosensitive types. At 36°C, the different penetration forces between primed and nonprimed seeds were compared after the first hour of imbibition and the last hour before radicle protrusion. The force required to penetrate the achene was affected by genotype, seed priming, and duration of imbibition. Puncture force declined as imbibition time increased in primed and nonprimed seed of all genotypes except 'Dark Green Boston'. The force required for penetration of the endosperm in nonprimed seeds of 'Dark Green Boston' increased with time and that for primed seeds was reduced. In 'Valmaine', penetration force remained similar in both primed and nonprimed seeds. In the three thermotolerant genotypes, penetration force decreased in primed and nonprimed seeds during imbibition. Priming lowered the initial force necessary to penetrate the achene and endosperm. Thermotolerant genotypes or seeds that were primed germinated at 36°C. For this process to occur, first, there must be a decrease in the resistance of the endosperm layer and, second, the pericarp and integument must be sufficiently weakened that tissue resistance is lower than the turgor pressure of the expanding embryo. The resistance decreased as thermotolerance increased, and priming enhanced the decrease in resistance among thermosensitive genotypes.

CHAPTER 5
STRUCTURAL CHANGES IN LETTUCE SEED DURING GERMINATION ALTERED BY GENOTYPE, SEED MATURATION TEMPERATURE, AND PRIMING

Introduction

Germination of lettuce seed is inhibited by high temperatures in the form of a barrier by the three tissues surrounding the embryo of lettuce seed, namely the pericarp, integument, and endosperm (Borthwick and Robbins, 1928). The pericarp is a ribbed structure consisting of thick-walled, lignified cells. The integument is composed of the remnants of the outer epidermis and some parenchymous cells. The endosperm is two cells thick, except at the radicle end where it is three or more cells thick. The cell wall of the endosperm is thick and has numerous column-like protuberances (Borthwick and Robbins, 1928). At high temperature, these tissues, especially the endosperm layer, act as a physical barrier, interfering with lettuce seed germination (Ikuma and Thimann, 1963b; Nabors and Lang, 1971; Speer, 1974).

The major biochemical events that occur in seed germination of lettuce include the activation of enzymes, mobilization of stored reserves and cell wall materials, and decrease in endosperm weight (Psaras et al., 1981). Cell-wall-degrading enzymes located in the micropylar region of the endosperm tissue are activated during imbibition and this induction may be a necessary prerequisite for normal germination. There is abundant evidence for this hypothesis: a) the increase in germination produced by cutting the endosperm tissue (Ikuma and Thimann, 1963b), b) the buckling of the embryo within an unweakened endosperm (Pavlista and Haber, 1970), c) disruptions of the endosperm tissue in the micropylar region prior to the onset of germination (Pavlista and Valdovinos, 1978), d) the increase in activity of carboxymethylcellulase prior to

endosperm degradation (Pavlista and Valdovinos, 1975), and e) the injection of cellulase and pectinase under the endosperm envelope, inducing significant germination (Ikuma and Thimann, 1963b). While there are several researchers who have worked on the analysis of these enzymes, the identification of the cell-wall-degrading enzyme(s) is still in question (Bewley and Halmer, 1980/81; Dutta et al., 1994; Halmer et al., 1976; Ikuma and Thimann, 1963b; Ouellette and Bewley, 1986).

Pavlista and Valdovinos (1978) used scanning electron microscopy to observe the disruption of the endosperm of 'Grand Rapids' lettuce seed before germination. Cracks and pits along the walls and surface of the endosperm cells were evident, and the tip of the micropylar region of some achenes had large breaks. Endosperm disruption was also determined in imbibed achenes at 12 hours. Endosperm weakening was enhanced in light-treated seeds and the cytoplasm of the mycropylar endosperm cells underwent drastic changes. Even though the cell walls of the entire endosperm remained intact, the cells became highly vacuolated and reserve materials (protein and lipid) were mobilized (Psaras et al., 1981).

Priming circumvents thermoinhibition in lettuce seed by shortening the germination period, especially at phase II. Enzyme and metabolic activity are enhanced during seed priming and are related to increasing seed vigor during germination (Khan et al., 1978). It is also possible that priming affects key metabolic processes related to the breakdown of protein and lipid reserve materials (Khan et al., 1980/81). This degradation of stored reserves may increase the ability of the primed seeds to absorb water, to germinate rapidly, and to tolerate a stressful environment (Khan, 1992).

Puncture tests have been conducted to analyze the role of the physical barrier presented by seed coverings during germination of pepper (Watkins and Cantliffe, 1983), tomato (Groot and Darssen, 1987), muskmelon (Welbaum et al., 1995), and lettuce (Tao and Khan, 1979). These results suggested that various parts of the seed coverings played a major role in controlling germination. Seed priming appears to

induce the process of weakening of the perisperm in muskmelon (Wilson and Welbaum, 1994) or the endosperm of lettuce (Chapter 4). Priming led to a lowering of the initial force necessary to penetrate the seed coverings, especially the endosperm, and this reduction was enough to overcome the resistance of seed coverings when germination took place at high temperature.

In lettuce seed, the mechanism that results in increased embryonic growth or weakening of the endosperm is still unknown. Lettuce genotypes with higher levels of thermotolerance can be selected, and the ability of the seed to germinate at high temperature can be enhanced by the environment during seed production. The primary objective of this research was to identify the ultrastructural alterations in the micropylar area of lettuce seed during germination as the seed overcomes thermoinhibition.

Materials and Methods

Materials

Two thermosensitive ('Dark Green Boston' and 'Valmaine') and three thermotolerant ('Floricos 83', 'Everglades', and PI 251245) genotypes were used, as described in Chapter 3.

Seeds of the five genotypes that were produced in the Salinas area of California in 1992 were primed using polyethylene glycol (PEG), employing the priming method as described in Chapter 4. Priming conditions were imposed as shown in Table A-5.

Seeds of 'Dark Green Boston', 'Valmaine', and PI 251245 were produced in growth chambers at 20/10° and 30/20°C. The method of seed production is described in Chapter 3.

Germination Tests

Primed and nonprimed seeds and seeds matured at 20/10°C and 30/20°C were germinated at 36°C in 12/12 hours light/dark periods. Thirty seeds per replication per treatment were placed in a 5.5 cm Petri dish with two layers of 4.5 cm diameter Whitman #3 filter paper moistened with 1-2 ml of distilled water. Distilled water was added as needed to keep the filter paper moist. Germination was defined as visible radicle protrusion through the pericarp and total percent germination was calculated. All treatments had three replications.

Sectioning

Eighty primed and nonprimed seed of all five genotypes were placed on moist filter paper in Petri dishes and incubated for various periods in a12/12 hours light/dark photoperiod at 36°C. After a prescribed time, the imbibed seeds were cut in half and the micropylar region was fixed in Trump solution (10 cc of FAA + 0.2% glutaraldehyde in 0.5M phosphate buffer at pH 7.2. McDowell et al., 1976) and stored for later use. Two sampling times per genotype were undertaken: right before radicle emergence and when close to 30% radicle protrusion. Sampling hours are listed in Table A-8. Eighty seeds each from 'Dark Green Boston', 'Everglades', and PI 251245 matured at 20/10° and 30/20°C were treated in the same manner as described above.

Fixed seeds were rinsed three times with 0.1M sodium cacodylate buffer at pH 7.2 for 10 min at room temperature, dehydrated in a graded ethanol series (10 min each step) followed by 10 min in 100% acetone twice, then embedded in a LR White resin (London Resin Company) series for 4 days. Polymerization of LR White resin was carried out at 52-57°C for two days (Hall and Hawes, 1991). Sections 1μm thick were cut with a glass knife on a LKB 8800 ultratome III (LKB Bromma) and were routinely stained in 0.5% azure II + 0.5% methylene blue. Six primed seeds and eight seeds

matured at each of the two temperatures were photographed using a light microscope (Nikon, microflex HFX).

Endosperm cells in the micropylar area of 'Dark Green Boston' and Everglades' seeds matured at 20°/10°C and 30°/20°C were counted under a light microscope. Four sections were used from seeds matured at 20°/10°C or three from seeds matured at 30°/20°C. Different numbers of seed sections were observed because the endosperm cells of seed matured at 30°/20°C shrank or dissolved, making it extremely difficult to obtain perfect enough sections to count the endosperm cell numbers in the micropylar area. Three observations were photographed of each seed section using a light microscope (Nikon, microflex HFX).

Results

Seed Structure

The basic structure of a lettuce achene is illustrated in Figure 5-6a,b. The tissues that surround the embryo of the achene are the pericarp, integument, and endosperm (Borthwick and Robbins, 1928). The endosperm tissue is connected to the integument tissue. Near the micropylar end, a constriction of the endosperm membrane connects with a cap structure that is part of the pericarp and ends in a distinct stalklike structure that is the base of the pappus.

Structural Alterations in Primed Seeds

At two different imbibition hours, depending on germination time for the various genotypes, structural alterations of the endosperm tissue of primed and nonprimed seed were observed at the micropylar area of the achene, the region of radicle protrusion.

'Dark Green Boston'. At 36°C, primed seed had 86% germination (time to 50% of final seed germination was 16 hours) but nonprimed seed did not germinate (Appendix Table 6). After primed and nonprimed seeds were imbibed for nine hours, a crack appeared on one side of the cap tissue of the pericarp and integument (Figure 5-1a,b,c). Endosperm cells of nonprimed seed were round and had small vacuoles spread throughout the cytoplasm (Figure 5-1b).

The radicle protruded through the seed coverings after nine hours of imbibition in primed seed (Figure 5-1d). The embryo of primed seed was closely enveloped by the endosperm layers and cell size of the inner layer (close to the embryo) of endosperm appeared to be thinner than the cells of the outer layer (close to the integument) (Figure 5-1e).

After primed seeds were imbibed for 13 hours, the endosperm layer had become thinner and the radicle had pushed through this layer (Figure 5-2a,c,d). A break in the endosperm tissue in front of the radicle was noted (Figure 5-6b,f) and the disappearance of cell walls of the endosperm tissue was noticeable (Figure 5-6e).

After nonprimed seeds were imbibed for 13 hours, the cytoplasm in the micropylar area appeared condensed. In the inner layer of the endosperm tissue, the shape of the cells was flat, while at of the outer layer the cells were still round. Cell walls in the outer layer were thicker than those in the inner layer (Figure 5-3a-d). After having been imbibed for 13 hours, the uncondensed cytoplasm in the endosperm cell could be observed (Figure 5-3e).

'Valmaine'. At 36°C, primed seed germinated 85% (time to 50% of final seed germination was 12 hours) and nontrimmed seed failed to germinate (Appendix, Table 6). After primed seeds were imbibed for five hours, a crack appeared on one side of the cap tissue (Figure 5-4a,b). The endosperm layer separated from the integument, sank, and moved close to the embryo (Figure 5-4a). After 12 hours, the growing embryo was

Figure 5-1. Longitudinal sections of 'Dark Green Boston' lettuce seed. Each bar=30μm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(a) Nonprimed seed imbibed for nine hours: crack on one side of cap tissue.

(b) Nonprimed seed imbibed for nine hours: higher magnification of the micropylar area of (a).

(c) Primed seed imbibed for nine hours: crack on one side of cap tissue; embryo enveloped by thin endosperm which connects with the cap tissue (arrow).

(d) Primed seed imbibed for nine hours: thin layer endosperm tissue.

(e) Primed seed imbibed for nine hours: higher magnification of the endosperm layer of (d) (arrow).

C: crack; Cs; cap structure; Ed: endosperm.

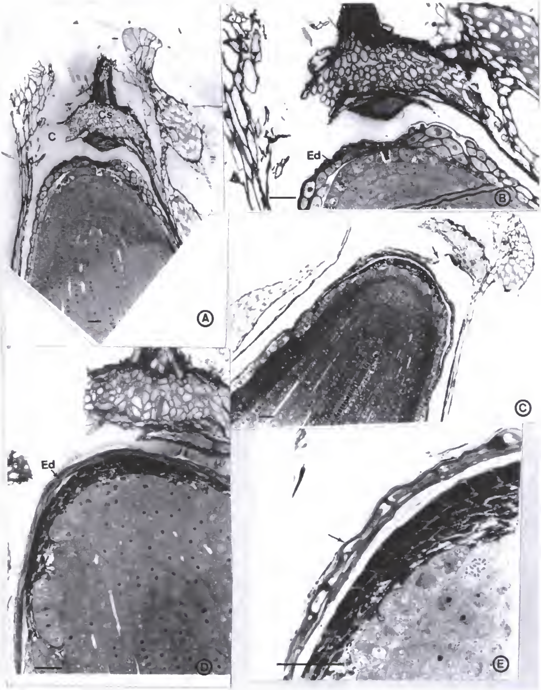

Cs
C
Ed
B
A
C
Ed
D
E

Figure 5-2. Longitudinal sections of 'Dark Green Boston' primed seed imbibed for 13 hours.
Each bar=30μm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(a) Embryo enveloped by the disrupted, thin endosperm (arrow).

(b) Disruption in the endosperm layer (arrow).

(c) Crack on one side of cap tissue; embryo enveloped by the thin endosperm.

(d) High magnification of the radicle protrusion.

(e) High magnification showing the dissolution of the outer, inner, and lateral walls in the layer of the endosperm cells (arrows).

(f) High magnification of dissolved endosperm layer (arrow).

C:crack; Em:embryo; R:radicle.

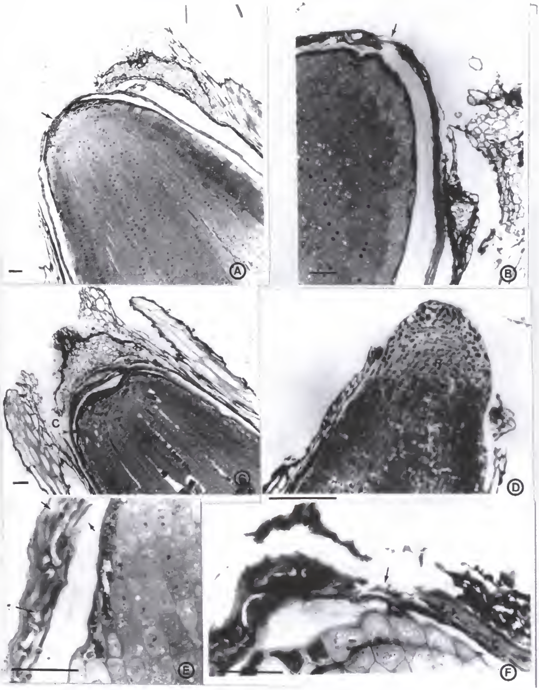


Figure 5-3 Longitudinal sections of 'Dark Green Boston' seed.
Each bar=30μm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(a) Nonprimed seed imbibed for 13 hours: dense cytoplasm in the endosperm cells (arrow).

(b) Nonprimed seed imbibed for 13 hours: dense cytoplasm in the endosperm cells.

(c) Nonprimed seed imbibed for 13 hours: higher magnification of endosperm layer of (a) (arrow).

(d) Nonprimed seed imbibed for 13 hours: higher magnification of endosperm layer of (b) (arrow).

(e) Primed seed imbibed for 13 hours: crack has appeared and cytoplasm in the endosperm cells has not condensed.

Ed: endosperm.

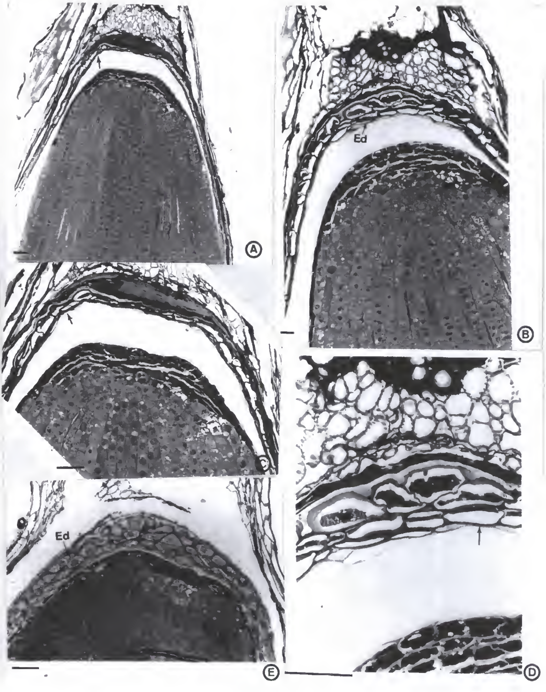

Ed
A
B
C
Ed
E
D

Figure 5-4. Longitudinal sections of 'Valmaine' seed.
Each bar=30µm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(a) Primed seed imbibed for five hours: edosperm has separated from the integument and close to the embryo.

(b) Primed seed that imbibed for five hours: crack has appeared.

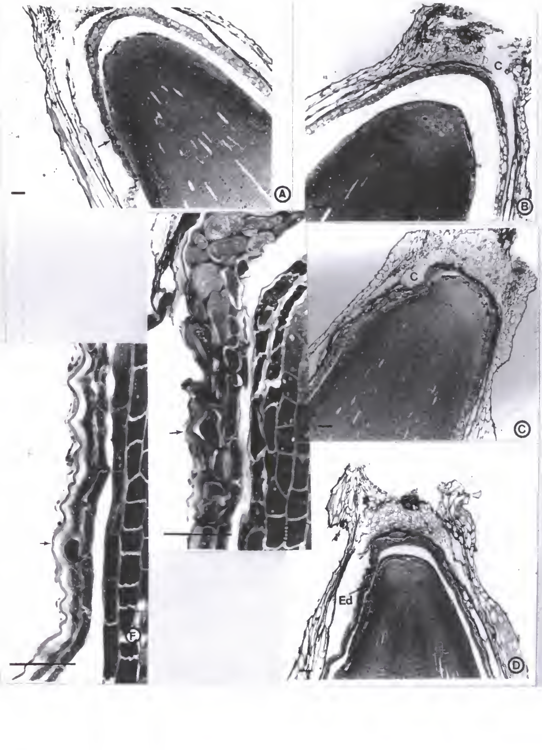
(c) Primed seed imbibed for 12 hours: crack has appeared.

(d) Nonprimed seed imbibed for 12 hours: wrinkled endosperm layer has separated from the integument and close to the embryo.

(e) Nonprimed seed imbibed for 12 hours: higher magnification of the endosperm layer of (d) (arrow).

(f) Nonprimed seed imbibed for 12 hours: higher magnification of the endosperm layer of (d) (arrow).

C:crack; Ed:endosperm.


A
B
C
c
c
D
Ed
F

surrounded by the endosperm layer. Part of the endosperm layer in the micropylar area separated from the integument and pericarp (Figure 5-4c).

After nonprimed seeds were imbibed for 12 hours, the structure of the endosperm cell walls still kept its integrity, even though the cell walls had already separated from the integument and were very close to the embryo (Figure 5-4d,e,f).

'Floricos 83'. In the median longitudinal section of the micropylar area in nonprimed seeds, there was a clear constriction of the endosperm membrane. A phloem strand connected to the integument and the cap tissue of the pericarp was observed (Figure 5-5a,b).

At 36°C, germination of primed seed was 100% (time to 50% of final germination was 12 hours) and that of nonprimed seed was 23% (time to 50% of final seed germination was 38 hours) after six days of imbibition (Appendix, Table 6). After primed and nonprimed seeds were imbibed for five hours, a crack appeared in one side of the cap tissue (Figure 5-5c,d). The endosperm layer in the cracked area separated from the integument and was close to the embryo. In primed seed after six hours of imbibition, dense cytoplasm appeared in some endosperm cells and the vacuoles disappeared (Figure 5-5e). In nonprimed seed after 12 hours of imbibition, a crack appeared in the pericarp tissue, but the integument did not break (Figure 5-5f).

'Everglades'. Germination of seeds of primed 'Everglades' was 100% (time to 50% of final seed germination was 12 hours) and for nonprimed 95% (time to 50% of final seed germination was 26 hours) at 36°C (Appendix, Table 6). Some radicles of primed and nonprimed seeds protruded after six and 12 hours of imbibition, respectively. After primed and nonprimed seeds were imbibed for five hours, a crack appeared on one side of the cap tissue in the micropylar region (Figures 5-6a, 5-7a,b), with the gap in primed seed larger than that in nonprimed seed. This crack or gap was an opening for the endosperm and embryo tissues.

Figure 5-5. Longitudinal sections of 'Floricos 83' seed.
Each bar=30μm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(a) Nonprimed seed imbibed for five hours: the phloem strand connects the endosperm and cap tissue.

(b) Nonprimed seed imbibed for five hours: higher magnification of the phloem strand and endosperm layer of (a).

(c) Primed seed imbibed for five hours: crack has appeared on one side of the cap tissue and the shrunk endosperm layer close to the embryo (arrow).

(d) Nonprimed seed imbibed for five hours: crack has appeared on one side of the cap tissue.

(e) Primed seed imbibed for six hours: dense cytoplasm in some endosperm cells (arrow).

(f) Nonprimed seed imbibed for 12 hours: the pericarp has broken but the integument did not break; cytoplasm has condensed in some endosperm cells (arrow).

C:crack; Cs:cap structure; Ed:endosperm; Ps:phloem strand.

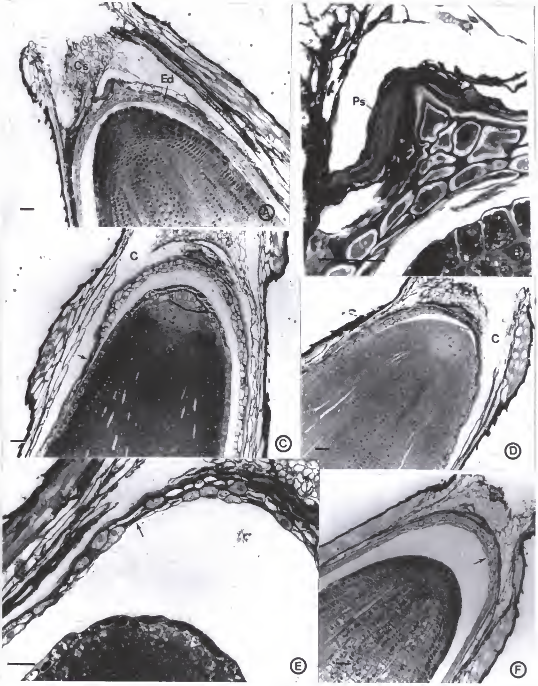

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Figure 5-6. Longitudinal sections of 'Everglades' nonprimed seed imbibed for five hours.
Each bar=30μm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(a) Endosperm cells four cells deep in the central mycropylar area.

(b) Endosperm cells two cells deep in the central mycropylar area.

Pc: pericarp; It: integumernt; Cs: cap structure; Ed: endosperm; Em: embryo.

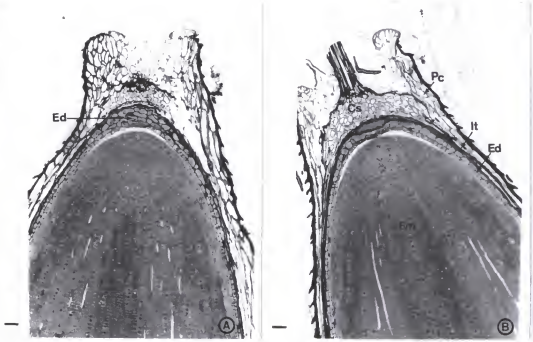
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Figure 5-7. Longitudinal sections of 'Everglades' primed seed imbibed for five hours. Each bar=30μm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(a) Crack has appeared and endosperm layer has separated from the integument.

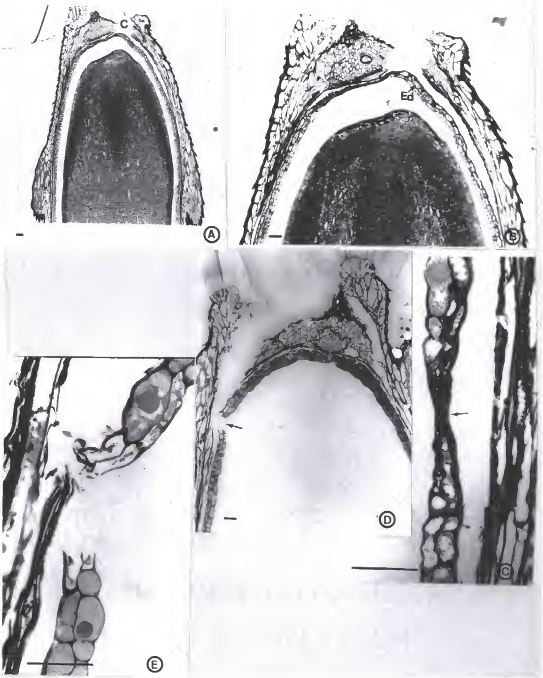
(b) Higher magnification of the endosperm layer of (a).

(c) Shrunken endosperm cells (arrow).

(d) Ruptured endosperm layer (arrow).

(e) Higher magnification of the ruptured endosperm layer.

C: crack.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In primed seeds, there was a weak, narrow region in the loosened endosperm tissue (Figure 5-7c). Around this region, the endosperm cells had a dense cytoplasm, no vacuole, and were shrunken (Figure 5-8d). A rupture of the endosperm layer was visible in Figures 5-7d,e and 5-8a,b,c. In some endosperm cells, the inner cell wall had ruptured and the cytoplasm had flowed into the space between the endosperm and embryo (Figure 5-8e,f). After primed seeds were imbibed for six hours, the endosperm tissue had the same structural alterations as those imbibed five hours (Figure 5-9a-d).

After being imbibed for 14 hours, the growing radicle of nonprimed seeds was closely enveloped by the endosperm layer, which became narrow and wrinkled but remained integrated (Figure 5-9e,f). An opening on one side of the pericarp and integument appeared and the embryo had grown toward this opening.

PI 251245. Germination of primed seeds was 100% (time to 50% of final seed germination was 12 hours) and nonprimed seeds was 93% (time to 50% of final seed germination was 17 hours) at 36°C (Appendix, Table 6). The radicle of primed seeds protruded after four hours of imbibition. A comparison was made of the median longitudinal sections of the micropylar area of the nonprimed seeds after having been imbibed 17 hours (Figure 5-10a) and primed seeds after being imbibed three hours (Figure 5-10b). In nonprimed and primed seeds after imbibition, the cytoplasm of the endosperm cells was condensed in the open (cracked) area. The endosperm cells on the lateral side had numerous small vacuoles containing protein bodies and the cell walls of the outer cells were thicker than those of the inner cells (Figure 5-10e). In primed seed, the cell wall of the endosperm layer had already ruptured (Figure 5-10c,d).

After primed seeds were imbibed for three hours, a disruption of the endosperm cells in the gap area was visible (Figure 5-11a-d). The cytoplasm of the disrupted cells was not dense, and some vacuoles and protein bodies were present although cell walls had ruptured and the cellular content had disappeared. Nevertheless, intact endosperm tissue could be observed in some seeds (Figure 5-11e).

Figure 5-8. Longitudinal sections of 'Everglades' primed seed imbibed for five hours. Each bar=30µm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(a) Ruptured endosperm layer (arrow).

(b) Ruptured endosperm layer (arrow).

(c) Embryo grown through ruptured endosperm tissue (arrow).

(d) Higher magnification of dense cytoplasm in the shrunken endosperm layer of (b) (arrow).

(e) Higher magnification of the ruptured endosperm layer of (c) (arrow).

(f) Higher magnification of the ruptured endosperm layer of (c) (arrow).

Em: embryo.

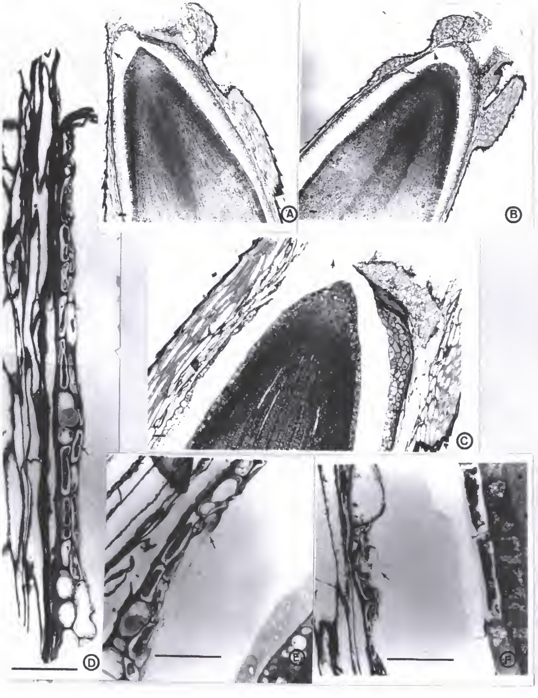
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Figure 5-9. Longitudinal sections of 'Everglades' seed.
Each bar=30μm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(a) Primed seed imbibed for six hours: endosperm layer has sunk (arrow).

(b) Primed seed imbibed for six hours: crack has appeared and endosperm tissue has sank (arrow).

(c) Primed seed imbibed for six hours: shrunken endosperm cells of (b) (arrow).

(d) Primed seed imbibed for six hours: ruptured endosperm (arrow).

(e) Nonprimed seed imbibed for 14 hours: embryo growing toward the opening in the pericarp and integument (arrow).

(f) Nonprimed seed imbibed for 14 hours: growing embryo enveloped by the endosperm layer.

Ed: endosperm; Em: embryo.

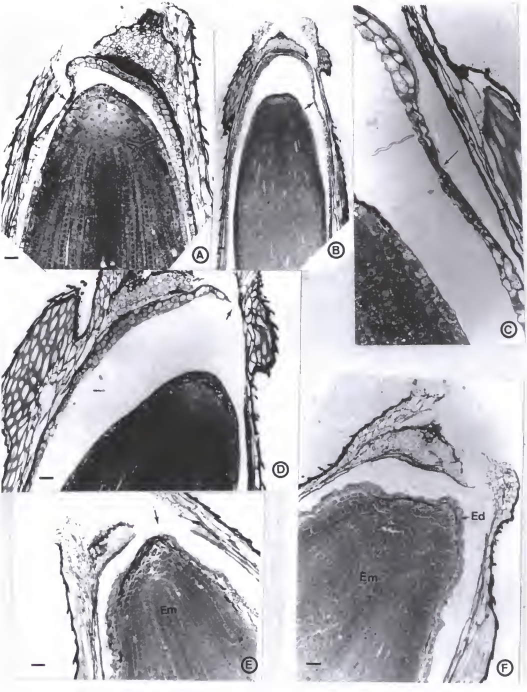
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Figure 5-10. Longitudinal sections of PI 251245 seed.
Each bar=30μm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(a) Nonprimed seed imbibed for 17 hours: crack has appeared and dense cytoplasm is present in this area (arrow).

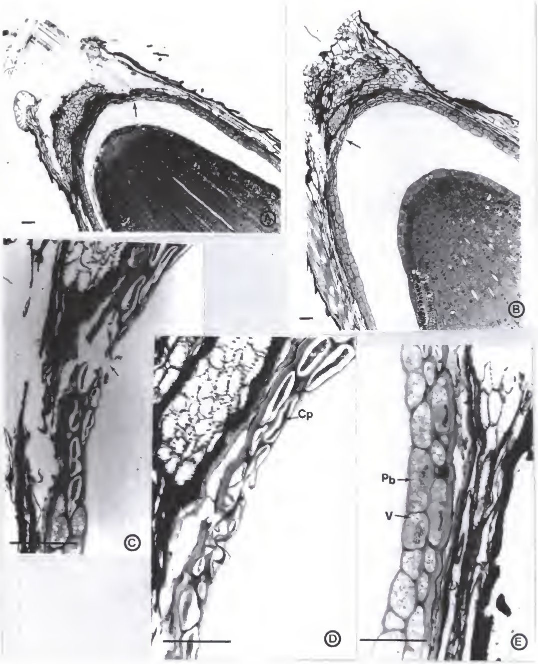
(b) Primed seed imbibed for three hours: ruptured endosperm layer (arrow).

(c) Primed seed imbibed for three hours: higher magnification of the ruptured endosperm layer of (b) (arrow).

(d) Primed seed imbibed for three hours: higher magnification of the ruptured endosperm layer and the cytoplasm has condensed.

(e) Primed seed imbibed for three hours: lateral side endosperm tissue with numerous vacuoles and protein bodies.

C: crack; Cp:cytoplasm; Cw: cell wall; Pb: protein body; V: vacuole.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Cp
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Figure 5-11. Longitudinal sections of PI 251245 primed seed imbibed for three hours. Each bar=30μm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

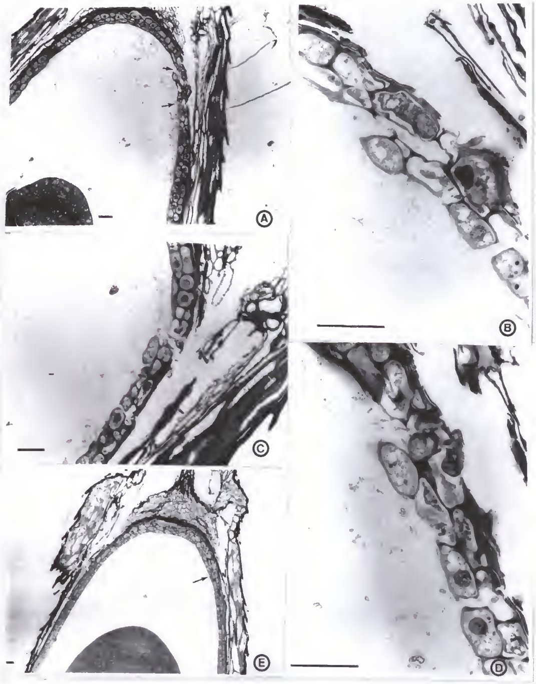
(a) Ruptured endosperm cell layer (arrows).

(b) Higher magnification of the ruptured endosperm layer of (a).

(c) High magnification of the ruptured endosperm layer.

(d) Higher magnification of the ruptured endosperm layer of (c).

(e) Intact endosperm tissue (arrow).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In primed seeds imbibed for three hours (Figure 5-12a,b) and four hours (Figure 5-12c,d), the endosperm cells were disrupted and had dense cytoplasm. Rupture of the endosperm cell wall also was observed.

Endosperm Cells

Cell walls of endosperm cell were thick, especially on the outer side of the endosperm layer, similar to that described by Pasaras et al. (1981). Additionally, protein bodies were observed in the vacuoles. During seed germination, structural alterations were observed. Some of those changes are described below.

After nonprimed 'Dark Green Boston' seeds were imbibed for nine and 13 hours, the cytoplasm of the lateral endosperm cells had gradually condensed (Figure 5-13a,b). Primed seeds imbibed for nine hours had denser cytoplasm, flatter shape, and thinner cell walls compared to nonprimed seeds (Figure 5-13c).

Primed seed of 'Floricos 83' had two different types of endosperm cells: swollen, oval cells in which several protein bodies were present, and flat cells with dense cytoplasm where the protein bodies had disintegrated and the vacuoles had broken down (Figure 5-13d).

Comparison of the two different types of endosperm cells in 'Everglades' primed seed revealed huge vacuoles which confined the cytoplasm to a thin layer pressed against the cell wall (Figure 5-13e). Some protein bodies were present in the vacuoles. In Figure 5-13f, the cytoplasm had gradually condensed around the shrunken vacuoles in which some protein bodies were evident.

Structural Alterations in Seeds Matured at Two Temperatures

'Dark Green Boston'. Germination at 36°C of seed matured at 20°/10°C was 2% (time to 50% of final seed germination was 24 hours) and for seed matured at 30°/20°C, it was 74% (time to 50% of final seed germination was also 17 hours) (Appendix, Table

Figure 5-12. Longitudinal sections of PI 251245 seed.
Each bar=30µm; i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(a) Primed seed imbibed for three hours: ruptured endosperm layer (arrow).

(b) Primed seed imbibed for three hours: higher magnification of the ruptured endosperm layer of (a).

(c) Primed seed imbibed for four hours: ruptured endosperm layer.

(d) Primed seed imbibed for four hours: higher magnification of the ruptured endosperm layer of (c).

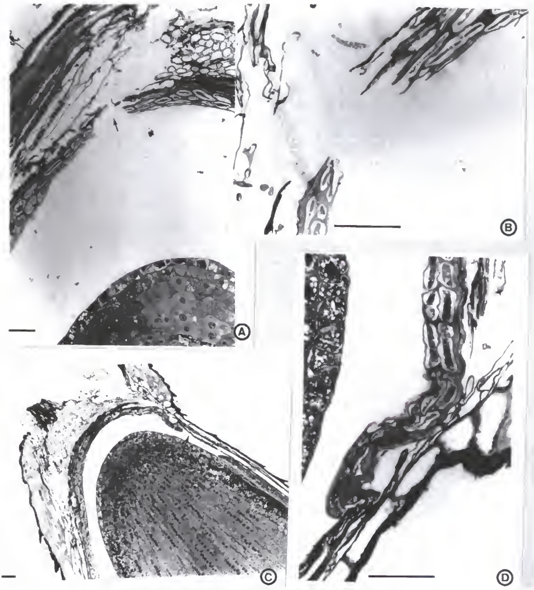
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Figure 5-13. The endosperm cells at the side of embryo.
Each bar=30μm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(a) Nonprimed seed of 'Dark Green Boston' after nine hours imbibition: numerous vacuoles and protein bodies in the endosperm cells (arrow).

(b) Nonprimed seed of 'Dark Green Boston' after 13 hours imbibition: densed cytoplasm has appeared.

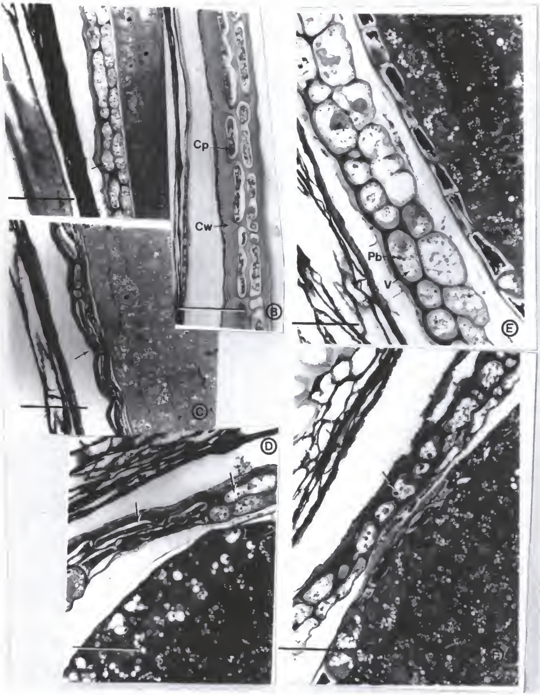
(c) Primed seed of 'Dark Green Boston' after nine hours imbibition: compared to (b), the cytoplasm was more condensed and endosperm cell walls were thinner (arrow).

(d) Primed seed of 'Floricos 83' after 16 hours imbibition: dense cytoplam and a large vacuole and protein bodies in different endosperm cells (arrows).

(e) Primed seed of 'Everglades' after five hours imbibition: huge central vacuole in endosperm cells.

(f) Primed seed of 'Everglades' after five hours imbibition: dense cytoplasm and large protein bodies in the shrunken cells (arrow).

Cw: cell wall; Cp: cytoplasm; Pb: protein body; V: vacuole.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9). After 10 hours of imbibition, seeds matured at 30°/20°C had above 30% radicle protrusion. A two-cell thickness of endosperm layer sank had separated from the integument (Figure 5-14a), and some of the endosperm cells had shrunk (Figure 5-14c). Cytoplasm of the endosperm cells had varying degrees of condensation (Figure 5-14 d, f,g). There were numerous small vacuoles containing protein bodies in the endosperm cells. The size of individual vacuoles increased over time and the cytoplasm gradually became condensed.

Normally, the central, thickest area directly in front of the radicle tip contained five endosperm cells, which were reduced to four, three, and two as one moved laterally from the center (Figure 5-3e). In 50% of the section of seeds that matured at a low temperature, the endosperm layers increased in thickness and the width of this area also increased (Figure 5-14b,e).

The endosperm layer of seeds matured at 30/20°C ruptured in the micropylar area after 10 hours of imbibition (Figure 5-15a,b,c). In endosperm cells, the cytoplasm appeared to have varying degrees of density (Figure 5-15d,e,f). In Figure 5-15g,h,i, the endosperm layer had ruptured at the thinnest point of the endosperm tissue. Some round cells on the lateral side of the endosperm layer had vacuoles containing protein bodies, and the inner cell wall appeared broken (Figure 5-15j,l). Cells on the lower lateral side of endosperm layer had a thick, integrated cell wall structure (Figure 5-15k).

<u>'Everglades'</u>. Germination at 36°C of seed matured at 20°/10°C was 50% (time to 50% of final seed germination was 18 hours) and for seed matured at 30°/20°C was 98% (time to 50% of final seed germination was also 18 hours) (Appendix, Table 9). After 10 hours of imbibition, the radicle of the seeds matured at the two temperatures protruded through the endosperm. The endosperm layer in the micropylar area had greater cell number and cell thickness for seeds matured at 20/10°C (Figure 5-16a,b,f,g). One side of the endosperm tissue had separated from the integument. In Figure 5-16c,d,e, endosperm cells in the micropylar area were in a thin layer compared

Figure 5-14. Longitudinal sections of 'Dark Green Boston' seed matured at 20/10°C. Each bar=30μm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(a) Crack has appeared and endosperm layer has separated from the integument (arrow).

(b) Thickening of endosperm layer in the mycropylar area.

(c) Shrunken endosperm cells (arrow).

(d) Condensed cytoplasm in various endosperm cells (arrow).

(e) Higher magnification of endosperm layer of (b).

(f) Condensed cytoplasm in various endosperm cells (arrow).

(g) Crack has appeared and cytoplasm has condensed in endosperm cells (arrow).

C: crack; Ed: endosperm.

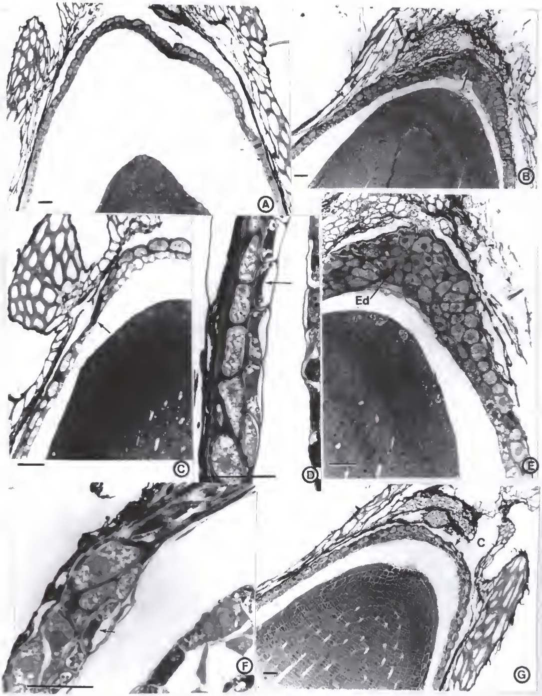
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Figure 5-15. Longitudinal sections of 'Dark Green Boston' seed matured at 30/20°C. Each bar=30μm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(a) Ruptured endosperm layer (arrow).

(b) Higher magnification of the ruptured endosperm layer of (a) (arrow).

(c) Higher magnification of the ruptured endosperm layer of (a) (arrow).

(d) Thinned endosperm layer in the mycropylar area (arrow).

(e) Condensed cytoplasm in various endosperm cells (arrow).

(f) Condensed cytoplasm and protein bodies in vacuoles in endosperm cells (arrows).

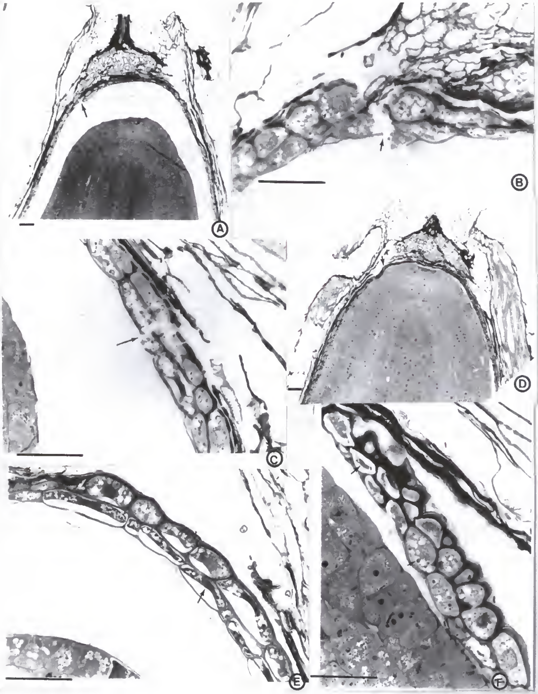

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Figure 5-15.(continued)
Longitudinal sections of 'Dark Green Boston' seed matured at 30/20°C.
Each bar=30μm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(g) Shrunken endosperm cells and ruptured endosperm layer (arrow) .

(h) Higher magnification of the ruptured endosperm layer of (g).

(i) Higher magnification of the ruptured endosperm layer of (g).

(j) Disrupted cell wall of endosperm cells on the lateral side of endosperm tissue (arrow).

(k) Thickening of cell wall in the outer and inner cells in the lower lateral side of endosperm tissue (arrow).

(l) Disrupted cell wall of endosperm cells in the lateral side of endosperm tissue (arrow).

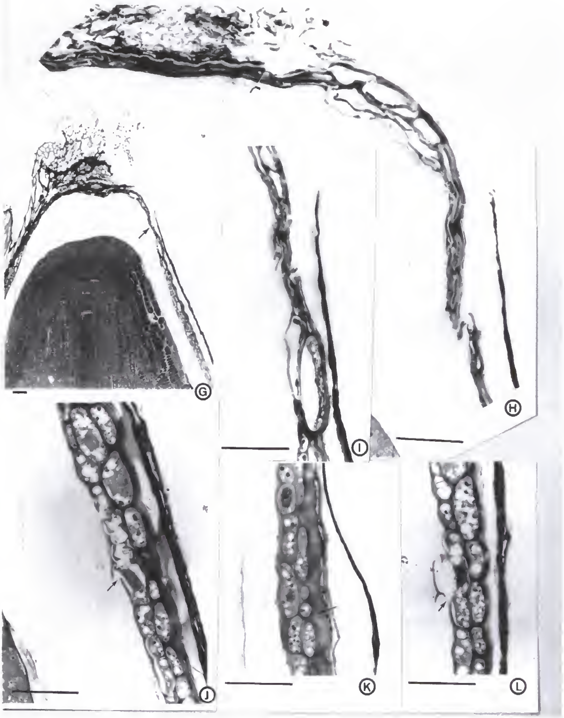
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Figure 5-16. Longitudinal sections of 'Everglades' seed matured at 20/10°C. Each bar=30μm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(a) Crack has appeared and the thick endosperm layer (arrow).

(b) High magnification of the dense endosperm cells.

(c) Crack has appeared and endosperm layer are shrunken and wrinkled (arrow).

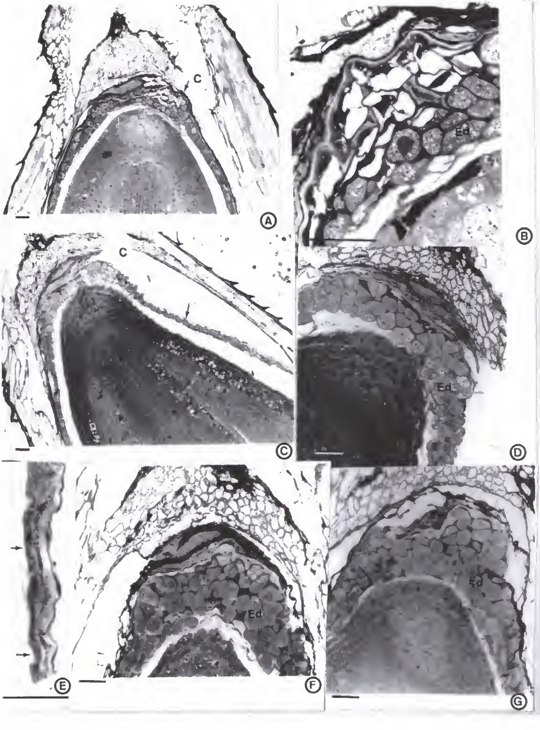
(d) Higher magnification of the endosperm cells in the mycropylar area of (c)

(e) Higher magnification of the shrunken endosperm cells of (c): dissolved inner side (arrows).

(f) Thickened endosperm layer in the mycropylar area.

(g) Thickened endosperm layer in the mycropylar area.

C:crack; Ed:endosperm.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to those at the top of the endosperm. In some cases, the endosperm layer of seeds matured at 20°/10°C had ruptured and the endosperm cells were disrupted (Figure 5-16h,i,j). In other cases, these seeds had flat endosperm cells with a thin layer at the micropylar area; the outer cell was was thicker and inner cell walls of some showed evidence of dissolution (Figure 5-16k,l,m), while in others part of the endosperm layer had shrunk (Figure 5-16n).

The endosperm cells of seeds matured at 30/20°C had large, round vacuoles and dense cytoplasm (Figure 5-17a,b,c,d). Part of the endosperm layer had separated from the integument and the cells in this region were flat with a dense cytoplasm (Figure 5-17e,f,g,h). The thin endosperm layer had separated from the integument (Figure 5-17i,j,k). One seed had the growing embryo enveloped by endosperm tissue containing intact cells (Figure 5-17l,m).

PI 251245. Germination of seed matured at 20/10°C was 33% (time to 50% of final seed germination was 26 hours) and for seed matured at 30°/20°C, it was 97% (time to 50% of final seed germination was 12 hours) at 36°C (Appendix, Table 9). Seed matured at 20°/10°C had endosperm tissue consisting of round cell and dense cytoplasm (Figure 5-18a,b). Part of the endosperm layer in the micropylar region had separated from the integument, and all cells contained dense cytoplasm (Figure 5-18c,d). In Figure 5-18e,f, the endosperm cell layer was disrupted in the micropylar region, but was still attached to the integument tissue.

All seeds matured at 30/20°C germinated at 36°C (Figure 5-19a,g). In one seed matured at 30°/20°C, the endosperm layer at the radicle end was totally disrupted, and the cell walls on the lateral side of the endosperm tissue were nearly broken after six to seven hours of imbibition (Figure 5-19b,c,d,e,f). In the remainder, the endosperm layer had separated from the integument (Figure 5-19h,i,j,k,l). As the radicle elongated, the endosperm cell walls seemed to dissolve or rupture.

Figure 5-16 (continued)
Longitudinal sections of 'Everglades' seed matured at 20/10°C.
Each bar=30μm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(h) Disrupted endosperm tissue.

(I) High magnification of the ruptured endosperm cells.

(j) High magnification of the ruptured endosperm cells.

(k) Shrunken endosperm tissue (arrow).

(l) Higher magnification of the dissolution endosperm cell walls of (k) (arrow).

(m) Higher magnification of the dissolution endosperm cells of (k) (arrow).

(n) Shrunken endosperm cells (arrows).

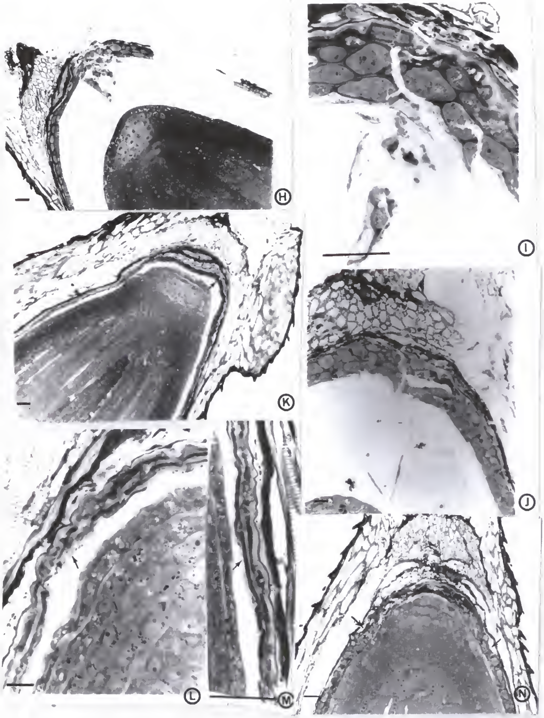
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Figure 5-17. Longitudinal sections of 'Everglades' seed matured at 30/20°C. Each bar=30μm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(a) Dense cytoplasm and nearly empty endosperm cells (arrow).

(b) Higher magnification of the nearly empty endosperm cells.

(c) Empty endosperm cells have sunk (arrow).

(d) Higher magnification of the very dense cytoplasm in endosperm cells of (c) (arrow).

(e) Part of endosperm cell layer has shrunken (arrow).

(f) High magnification of dense cytoplasm in the inner side of endosperm layer (arrow).

(g) Higher magnification of dense cytoplasm in the shrunken endosperm layer of (e) (arrow).

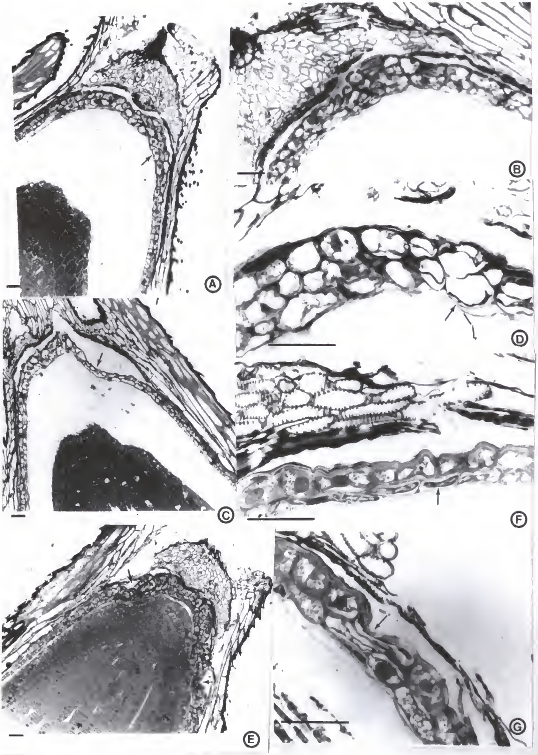
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Figure 5-17 (continued)
Longitudinal section of ‘Everglades’ seed matured at 30/20°C.
Each bar=30μm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(h) Crack has appeared (arrow).

(I) Thinned endosperm layer has sunk (arrow).

(j) Crack has appeared in cap area (arrows).

(k) Higher magnification of shrunken endosperm layer of (j) (arrows).

(l) Endosperm layer enveloping the embryo (arrow).

(m) Higher magnification of endosperm layer of (l).

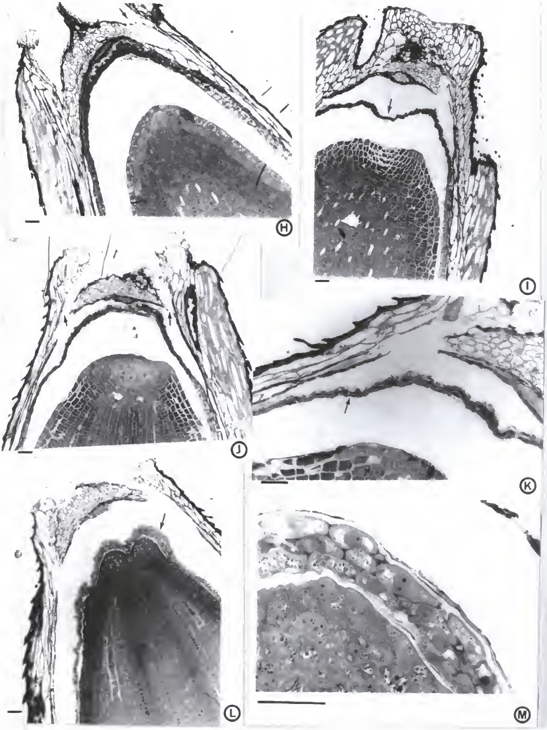
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Figure 5-18. Longitudinal sections of PI 251245 seed matured at 20/10°C. Each bar=30μm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(a) Dense cytoplasm in endosperm cells (arrow).

(b) Higher magnification of dense cytopasm in endosperm cells of (a)

(c) Thinned endosperm layer (arrow).

(d) Higher magnification of shrunken endosperm cells of (c) (arrow).

(e) Endosperm cells dissolution (arrow).

(f) Higher magnification of dissolved endosperm cells of (e).

Cp: cytoplasm; Ed: endosperm.

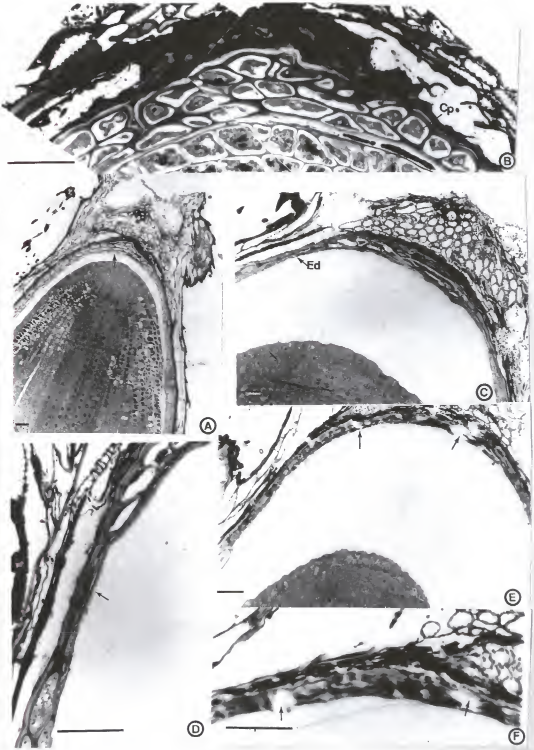
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Figure 5-19 Longitudinal section of PI 251245 seed matured at 30/20°C. Each bar=30μm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(a) Embryo growing toward the opening and disrupted endosperm tissue in the mycropylar area.

(b) Higher magnification of the disrupted endosperm cells of (a).

(c) Higher magnification of ruptured cell wall in the lateral side endosperm cells of (a) (arrow).

(d) Higher magnification of the disrupted endosperm cell wall in the lateral side endosperm cells of (a).

(e) Higher magnification of the disrupted endosperm cells in the lateral side endosperm cells of (a) (arrow).

(f) Higher magnification of the disrupted endosperm cells in the lateral side endosperm cells of (a) (arrow).

(g). Embryo growing toward the opening.

Em:embryo.


A
B
C
F
D
E
Em
G

Figure 5-19 (continued)
Longitudinal sections of PI 251245 seed matured at 30/20°C.
Each bar=30μm, i.e. 2.5mm=100x; 5mm=200x; 10mm=400; 25mm=1000x.

(h) Sunken endosperm layer.

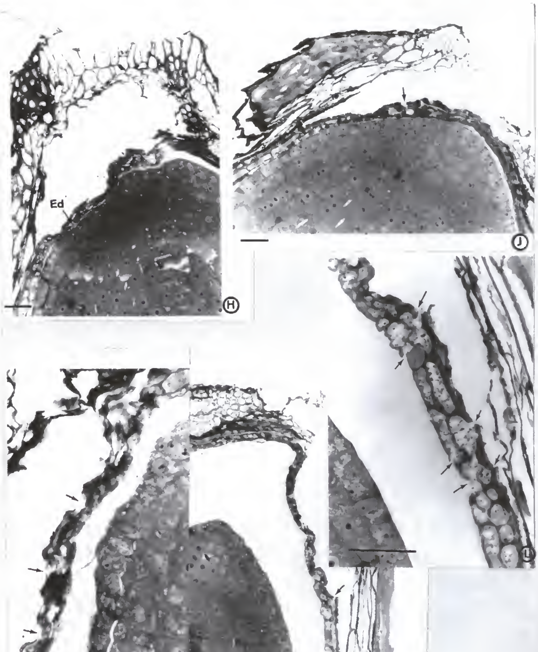
(i) Higher magnification of the dissolved endosperm cells of (a) (arrows).

(j) The integument and pericarp has broken (arrow).

(k) Ruptured and wrinkled endosperm cell layer (arrows).

(l) Higher magnification of the ruptured endosperm cell wall of (k) (arrows).

Ed: endosperm.


Ed
H
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K
L

Cell Number

The micropylar area of the endosperm layer was more than two cells thick. Endosperm cells in the micropylar area of 'Dark Green Boston' and 'Everglades' seeds matured at 20/10°C and 30/20°C were counted (Table 5-1). To do this, cells in each vertical layer, here termed a column, a vertical direction from inner side close to the embryo to the outer side close to the integument, were counted. Counting began from the number of endosperm cells increased from two and ended where the number of endosperm cells returned to two (Table 5-1).

Seeds of 'Dark Green Boston' matured at 20/10°C had more endosperm cells in front of the radicle compared to the seeds matured at 30/20°C. Cells increased not only in the number of cells in the column, but also in the number of columns more than two cells deep. For seeds matured at 20°/10°C, the average number of endosperm columns with more than two cells was 14 columns compared to seven for seeds matured at 30°/20°C. At the center of the micropylar end, where vertical cell number was greatest, the highest average number of cells for seeds matured at 20°/10°C was 4.7 and there were four columns that exceeded 3.3 cells in number. For seeds matured at 30°/20°C, the highest average was 3.3, and that was only found in one column centrally located at the micropylar end.

'Everglades' seeds matured at 20°/10°C and 30°/20°C had nearly the same number of columns containing more than two endosperm cells in the micropylar area. Seeds matured at 20°/10°C had five columns, with average cell numbers exceeding 3.5 in the thickest area. Seeds matured at 30°/20°C had no columns with average cell numbers exceeding 3.5.

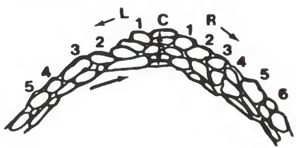
Table 5-1. Average endosperm cell numbers in the micropylar area of lettuce seeds matured at two temperatures.

		Column													
Genotype		L6[x]	L5	L4	L3	L2	L1	C	R1	R2	R3	R4	R5	R6	R7
Dark Green Boston	20/10°C	2.3	2.4	2.6	2.9	3.5	4.0	4.7	3.4	3.3	3.0	2.7	2.4	2.3	2.2
	±SE[z]	0.3	0.3	0.4	0.3	0.2	0.3	0.1	0.3	0.3	0.2	0.3	0.3	0.4	0.3
	30/20°C				2.3	3.0	2.8	3.3	2.8	2.8	2.3				
	±SE[y]				0.3	0.6	0.4	0.9	0.6	0.6	0.3				
Everglades	20/10°C		2.1	2.2	3.2	3.8	4.3	4.6	4.0	3.7	3.4	3.0	2.6	2.2	
	±SE[z]		0.1	0.3	0.1	0.4	0.6	0.2	0.2	0.3	0.3	0.1	0.3	0.3	
	30/20°C		2.0	2.9	3.1	3.2	3.1	3.4	3.1	3.1	3.0	2.8	2.4		
	±SE[y]		0.1	0.2	0.1	0.4	0.4	0.7	0.4	0.4	0.0	0.2	0.3		

[z] Values of means ±SE (Standard error) of four replications.

[y] Values of means ±SE (Standard error) of three replications.

[x] C: central column; L: left of C; R: right of C.



Discussion

Thermosensitivity was circumvented in lettuce seed by producing seed at temperatures of 30°/20°C or by seed priming (Chapters 3 and 4). Seed priming shortened the period from sowing to seedling establishment. During priming, several physiological and biochemical changes occur in seeds (Khan et al., 1980/81). When primed seeds are reimbibed, seed germination is rapid and uniform, so the restrictions to germination are bypassed at supraoptimal temperature (Khan, 1980). Mechanisms related to how priming increases embryonic growth or weakens the endosperm in lettuce seed have been studied by Bradford (1986), Cantliffe et al. (1984), and Karssen et al. (1989). Priming appears to lead to a reduction of the initial force necessary to penetrate the endosperm, integument, and pericarp (Chapter 4). The radicle protruded through the weakened area(s) of the seed. During priming, Guedes et al. (1981) viewed morphological changes in 'Minetto' lettuce seeds using a scanning electron microscope and reported that the membrane of endosperm cells gradually loosened. This loosening may be symptomatic of endosperm cell weakening and possibly was one of the results of priming that enhanced seed germination at high temperature.

Results of present anatomical studies indicated that structural alterations of the endosperm occurring in front of the radicle tip before radicle emergence were necessary for normal germination of lettuce achenes. The integument and pericarp did not appear to play major roles in restricting seed germination at high temperature since in all seeds these tissues split on one side of the micropylar area of the achene during imbibition, regardless of seed maturation temperature or priming. The endosperm layer directly in front of the radicle appeared to be the area of critical resistance to seed germination at high temperature.

The lettuce endosperm cell has a thick cell wall consisting primarily of polysaccharides: the micropylar walls were rich in arabinose and glucose, and the lateral walls were rich in galactomannan (Dutta et al., 1994). Protein bodies are present in the vacuoles of the endosperm cells and, in germinating seeds, the dissolution of the food reserves occurs first in the micropylar area (Bewley and Halmer, 1980/81). When this dissolution occurs, part of the endosperm layer in the micropylar region is weakened and ruptures. Other studies (Georghiou et al., 1983; Psaras et al., 1981), also indicated that, in germinating lettuce seed, the endosperm cells opposite the radicle were highly vacuolated prior to radicle protrusion and that storage materials appeared mobilized, although the endosperm cells in the lateral and cotyledonary areas remained intact.

The puncture tests and the anatomical studies presented here gave further evidence that the weakening of the endosperm tissue around the radicle tip prior to radical emergence was related to regulation of germination at high temperature. Puncture tests also indicated that, in thermosensitive lettuce genotypes, the force required to penetrate the endosperm increased at higher imbibition temperatures (i.e., 36°C). The level of puncture force resistance of the endosperm depended on genotype, and the timing of radicle emergence was controlled primarily by the rate at which the seed coverings weakened. A rupture in the loosened endosperm was noted sooner in primed seed of 'Everglades' and PI 251245 than in nonprimed counterparts. In both, the radicle elongated through this opening in the seed coverings. In primed seeds of 'Dark Green Boston' and nonprimed seeds of 'Everglades', the thin endosperm layer did not rupture, but closely surrounded the embryo. It may be that the endosperm resisted complete rupturing until the radicle forcefully penetrated through it, suggesting that the radicle may play a role in seed germination at supraoptimal temperature. For seed germination to occur, two mechanisms may be at work: one, that resistance of the endosperm may gradually weaken during the germination process until the endosperm finally ruptures; the other, that the endosperm layer had weakened, but not sufficiently to

spontaneously rupture, and germination could occur only when the force of the growing embryo increased sufficiently for the radicle to mechanically penetrate the endosperm.

Cantliffe et al. (1984) suggested that the osmotic potential of the lettuce radicle must be increased if cell elongation was to occur. In primed lettuce seeds, an increased accumulation of soluble amino nitrogen compounds and other hydrolytic products in the radicle tip could be a mechanism for overcoming thermodormancy (Khan et al., 1980/81; Takeba, 1983). In the radicle and cotyledons during lettuce seed germination, Srivastava and Paulson (1968) reported that the contents of protein bodies were depleted, and the protein bodies gradually became empty vacuoles. Takeba and Matsubara (1979) measured the embryonic growth potential, i.e., the osmotic potential of the embryonic axes, of 'New York' lettuce seed and demonstrated that intact seed could not germinate at 35°C because the growth potential was not enough to overcome the restraining force of the seed coat. In the present work, it was observed that the protein bodies in the endosperm cells dissolved during imbibition. Bewley and Halmer (1980/81) noted that this degradation of stored reserves in the endosperm diffused to the cotyledons and were hydrolyzed to small molecules which, presumably, were then absorbed by the cotyledons for use by the growing seedling. In primed seed, this degradation of endosperm cell reserves may enhance the ability of the radicle to absorb water, to germinate rapidly, and to tolerate stressful environments.

In this work, lettuce seed matured at 30°/20°C and 35°/25°C overcame thermoinhibition and germinated rapidly at 36°C for all genotypes except 'Valmaine' (Chapter 3). For seed to germinate rapidly, during the germination process the cell wall of the endosperm layer should rupture early. In this instance, during germination the endosperm cell structure changed in the micropylar area: the reserve protein was depleted and empty vacuoles appeared, the size of cells gradually decreased, the cytoplasm gradually condensed, and the endosperm cell walls in the radicle tip ruptured. The timing of the series of events between vacuolation of the endosperm cell and endosperm cell wall

disruption is not clear. For instance, for 'Dark Green Boston' and PI 251245 seeds matured at 30°/20°C, the broken cell wall could be observed before the cytoplasm had condensed. Although it is not clear what the first step is for enhancing the cell wall rupture, Gray et al. (1988b) suggested that the synthesis of cell-wall-weakening enzymes should have been activated during seed development and maturation. Further investigation is necessary to determine what critical enzymes are involved in weakening the endosperm cell or what processes occur that assure the function of hydrolytic enzymes in weakening the endosperm cell wall.

Seeds matured at 20°/10°C and 25°/15°C were larger than those that matured at 30°/20°C (Chapter 3). From viewing the sections, the endosperm layer of 'Dark Green Boston' and 'Everglades' matured at 20°/10°C had greater cell numbers and thickness in the micropylar area than seeds matured at 30°/20°C. Seeds matured at low temperature germinated poorly at 36°C. This poor germination may be related to the greater cell number in the micropylar area of the endosperm, where the thicker endosperm layer increased the mechanical resistance to germination at high temperature.

Using thermotolerant genotypes, producing seed at higher temperature, and employing seed priming, all produced similar effects for enhancing thermotolerance and all led to similar endosperm structural alterations during germination. This suggests that the mechanisms of each may be linked and that each variable helped to circumvent thermodormancy by releasing the physical resistance of the endosperm.

Summary

Seed genotype, maturation temperature, and priming all influenced thermotolerance in lettuce. To investigate thermotolerance in lettuce seed at high temperature, primed and nonprimed seed or seeds matured at 20°/10°C and 30°/20°C

were imbibed at 36°C. During seed germination, the structural changes of the seed coverings in front of the radicle tip were observed in an anatomical study. In all seeds during imbibition, regardless of seed maturation temperature or priming, a crack appeared on one side of the cap tissue and the endosperm separated from the integument in front of the radicle tip. Additional changes took place during imbibition: the protein bodies in the vacuoles enlarged and were gradually depleted, large empty vacuoles formed, the cytoplasm condensed, the endosperm shrank, the endosperm cell wall dissolved and ruptured, then the radicle elongated toward this ruptured area. The findings suggested that the papery endosperm layer presented mechanical resistance to lettuce seed germination and the weakening of this layer was a prerequisite to radicle protrusion at high temperature.

Seeds of 'Dark Green Boston', 'Everglades', and PI 251245 matured at 30°/20°C had greater thermotolerance than those matured at 20°/10°C. Results of the anatomical study indicated that the endosperm cell walls in front of the radicle of seeds matured at 30°/20°C were more easily disrupted and ruptured during early imbibition than seeds matured at 20°/10°C, suggesting that these seeds could germinate quickly at supraoptimal temperatures. From anatomical studies conducted to identify and characterize thermotolerance in lettuce seed germination, it was observed that genotype thermotolerance had the ability to reduce physical resistance of the endosperm by weakening the cell wall and by depleting stored reserves.

APPENDIX

Table A-1. Germination percentage of 21 lines of lettuce seeds matured under four temperatures and germinated over a temperature range of 20° to 38°C in dark.

	Germination temp (°C)						
Day/night temp.(°C)	20	24	27	30	33	35	38
			68288 (T1000)				
20/10	100	100	89.3	81.7	40	5.3	4
25/15	100	100	100	66.7	66.7	0	0
30/20	100	100	97.3	70.7	20	10.6	2.6
35/25	100	100	97.3	82.7	38.7	1.3	0
			T1008(Mussett)				
20/10	98.7	58	2.7	1.3	0	0	0
25/15	98.7	62.7	8	0	0	0	0
30/20	100	100	56	2.7	0	0	0
			T1008(Mussett)				
20/10	100	77.3	2.7	1.3	0	0	0
25/15	100	70.7	28	4	6.7	0	0
30/20	100	90.7	37.3	8	0.7	0	0
			T1011((Mussett)				
25/15	100	78.7	24	1.3	1.3	0	0
30/20	100	89.3	54.7	2.7	0	0	0
			T1013(Mussett)				
20/10	97.3	68	1.3	1.3	5.3	0	0
25/15	98.7	77.3	28	1.3	0	0	0
30/20	100	82.7	25.3	1.3	0.7	0	0
35/25	97.3	97.3	18.7	1.3	0.7	0	0
			T1018 (Valmaine)				
20/10	100	98.7	80	0	0	0	0
25/15	100	100	98.7	1.3	0	0	0
30/20	98.7	100	98.7	5.3	1.3	1.3	0
35/25	100	100	71.7	46.7	16.7	41.7	0
			T1019 (Valmaine)				
20/10	100	100	70.7	0	0	0	0
25/15	97.3	98.7	82.7	5.3	0	1.3	0
30/20	98.7	98.7	97.3	5.3	0	0	0
35/25	100	100	97.3	4	36	0	0
			T1020 (Valmaine)				
20/10	100	82	17.7	0	2.7	0	0
25/15	100	98.7	88	10.7	0	0	0
30/20	100	89.3	42.7	0.7	0	0	0
35/25	100	100	82	6	0	0	0
			T1021 (Valmaine)				
20/10	100.0	96.7	94.7	2.7	0.0	0.0	0.0
25/15	100.0	100.0	97.3	16.0	0.0	0.0	0.0
30/20	100.0	100.0	97.3	6.7	1.3	1.3	0.0
35/25	100.0	100.0	33.3	33.3	0.0	0.0	0.0
			T1027(Parris Island cos)				
20/10	100	87.3	73.3	8.3	0	0	0
25/15	98.7	98.7	85.3	9.3	2.7	0	0
30/20	98.7	98.7	94.3	1.3	0	0	0
35/25	100	100	100	29.3	33.3	33.3	0

Table A-1. (continued)

Germination percentage of 21 lines of lettuce seeds matured under four temperatures and germinated over a temperature range of 20° to 38°C in dark.

	Germination temp (°C)						
Day/night temp.(°C)	20	24	27	30	33	35	38
			T 1028 (Parris Island cos)				
25/15	100.0	100.0	96.0	9.3	0.0	0.0	0.0
30/20	100.0	100.0	92.0	16.0	0.0	0.0	0.0
35/25	100.0	100.0	100.0	14.0	14.0	4.7	0.0
			T 1029 (Parris Island cos)				
20/10	100.0	100.0	93.3	37.3	0.0	0.0	0.0
25/15	98.7	98.7	94.7	55.3	0.0	1.3	0.0
30/20	100.0	100.0	78.7	16.0	1.3	0.0	0.0
35/25	100.0	100.0	100.0	66.7	1.3	0.0	0.0
			T 1030 (Parris Island cos)				
20/10	100.0	96.0	69.3	4.0	2.7	0.0	0.0
25/15	100.0	100.0	98.7	58.7	0.0	0.0	0.0
30/20	100.0	100.0	98.7	9..3	0.0	0.0	0.0
			T 1036 (Tall Guzmaine)				
20/10	100.0	100.0	100.0	98.7	33.3	6.7	0.0
25/15	98.7	100.0	100.0	96.0	24.0	8.0	0.0
30/20	100.0	100.0	100.0	97.3	22.7	4.0	1.3
			T 1039 (Tall Guzmaine)				
20/10	100.0	100.0	100.0	98.7	90.7	17.3	5.3
25/15	100.0	100.0	98.7	100.0	34.0	18.7	5.3
30/20	100.0	86.7	80.0	57.3	8.0	0.0	0.0
			T1045 (Floricos 83)				
25/15	100.0	100.0	100.0	100.0	66.7	100.0	0.0
30/20	100.0	100.0	100.0	97.3	72.6	20.0	4.0
35/25	100.0	100.0	100.0	100.0	97.7	79.7	77.7
			T1047 (Floricos 83)				
25/15	100.0	100.0	100.0	100.0	100.0	66.7	0.0
30/20	100.0	100.0	100.0	93.3	38.7	17.3	0.0
35/25	100.0	100.0	98.7	98.7	89.3	74.7	40.0
			T1048 (Floricos 83)				
25/15	100.0	98.7	98.7	97.3	22.7	4.0	0.0
30/20	100.0	98.7	100.0	98.7	53.3	34.7	2.0
			T1049 (Floricos 83)				
30/20	100.0	100.0	100.0	98.7	17.3	6.7	1.3
35/25	100.0	100.0	98.7	100.0	92.0	69.3	42.7
			Dark Green Boston				
20/10	90.7	8.0	9.3	0.0	0.0	0.0	0.0
25/15	100.0	70.7	18.7	1.3	0.0	0.0	0.0
30/20	100.0	94.7	50.7	3.0	13.3	0.0	0.0
			T1131 (Foribibb)				
20/10	100.0	98.7	96.5	72.0	2.7	1.3	0.0
25/15	100.0	100.0	100.0	93.0	0.0	0.0	0.0
30/20	100.0	100.0	100.0	96.0	46.7	0.0	0.0
35/25	100.0	100.0	100.0	100.0	40.0	14.3	0.0

Table A-2. Germination percentage of lettuce seeds produced in Salinas, California and germinated over a temperature range of 20° to 38°C in dark.

	Germination temp.(°C)						
Genotype	20	24	27	30	33	35	38
Dark Green Boston	23	0	0	0	0	0	0
Everglades	100	100	70	3	0	0	0
Floribibb	100	100	100	30	0	0	0
Floricos 83	100	93	27	0	0	0	0
Mussette	93	0	3	0	0	0	0
Parris Island Cos	100	100	47	0	0	0	0
PI 251245	100	100	100	83	3	3	0
PI 68288	97	100	53	6	3	0	0
Tall Guzmaine	100	83	100	13	0	0	0
Valmaine	100	97	2	0	0	0	0

Table A-3. Analysis of variance for the percent germination of lettuce seeds matured under four temperatures.

		MS				
Source	df	Dark Green Boston	Everglades	Valmaine	Floricos 83	PI 251245
Germination temp.(GT)	3	1.155**	1.298**	5.858**	2.682**	0.291**
Linear	1	3.000**	0.703**	15.526**	6.953**	0.869**
Quadratic	1	0.277**	0.152**	0.0002NS	0.077*	0.004NS
Cubic	1	0.187*	0.037**	2.048**	1.014**	0.0005NS
Error a (Rep(temp))	8	0.022	0.009	0.023	0.009	0.019
Seed maturation temp.(SMT)	3	1.908**	0.0068**	0.278**	0.279**	0.538**
20 + 25°C vs 30+35°C	1	5.328**	0.385**	0.257**	0.600**	1.493**
20°C vs 25°C	1	0.257**	0.028*	0.561**	0.084*	0.036NS
30°C vs 35°C	1	0.139**	0.022*	0.019NS	0.155**	0.086*
SMT x GT	9	0.064**	0.023**	0.165**	0.112**	0.073**
Error b	24	0.014	0.004	0.016	0.019	0.017

*Data subjected to square root (arc sine) transformation.

Table A-4. Analysis of variance for the mean days to germination of lettuce seeds matured under four temperatures.

		MS				
Source	df	Dark Green Boston	Everglades	Valmaine	Floricos 83	PI 251245
Germination temp.(GT)	3	10.000**	3.379**	40.426**	2.682**	0.874**
Linear	1	26.334**	10.008**	109.62**	6.953**	2.604**
Quadratic	1	1.367**	0.105NS	0.563NS	0.077**	0.001NS
Cubic	1	2.301NS	0.025NS	11.094NS	1.014**	0.017NS
Error a (Rep(temp))	8	1.518	0.142	5.263	0.009	0.019
Seed maturation temp.(SMT)	3	36.557**	0.682*	2.077NS	0.279**	2.746**
20 + 25°C vs 30+35°C	1	89.927**	0.883*	3.308	0.600**	3.968**
20°C vs 25°C	1	19.44NS	0.546NS	2.042	0.084**	4.25**
30°C vs 35°C	1	0.304NS	0.617NS	0.882	0.155**	0.02NS
SMT x GT	9	0.724NS	0.204NS	1.52NS	0.112**	0.456**
Error b	24	1.032	0.148	3.103	0.019	0.113

Table A-5. The conditions for seed priming of five genotypes.

Genotype	Osmotic potential of PEG Mpa	Durations Days
Dark Green Boston	-1.2	4
Valmaine	-1.3	2
Floricos 83	-1.3	6
Everglades	-1.3	2
PI 251245	-1.3	2

Table A-6. Germination and time to 50% seed germination of primed and nonprimed seeds of lettuce genotypes at 36°C.

	Germination(%)		GT_{50} (hour)[y]	
Genotype	Primed	Nonprimed	Primed	Nonprimed
Dark Green Boston	86	0**[z]	16	0 **[z]
Valmaine	85	0**	12	0 **
Floricos 83	100	23**	12	38**
Everglades	100	95NS	12	26**
PI 251245	100	93**	12	17**

[z]NS, *, ** Nonsignificant or significant at P=0.05 or 0.01, respectively, by F-test.

[y] GT_{50}: Time to 50% of final seed germination.

Table A-7. Analysis of variance for mean force required to penetrate lettuce seed tissues during imbibition at 36°C

Dark Green Boston

		Achene		Endosperm	
Source	df	MS	Pr > F	MS	Pr > F
Hour	3	0.00025	NS	0.00002	NS
Erroe (Block(hour))	8	0.00034		0.00011	
Prime	1	0.00553	**	0.00089	*
Hour x Prime	3	0.00007	NS	0.00024	NS
Error b	8	0.00013		0.00009	

Valmaine

		Achene		Endosperm	
Source	df	MS	Pr > F	MS	Pr > F
Hour	3	0.00275	**	0.00003	NS
Error a (Block(hour))	8	0.00051		0.00017	
Prime	1	0.00045	NS	0.00000	NS
Hour x Prime	3	0.00019	NS	0.00000	NS
Error b	8	0.00019		0.00004	

Floricos 83

		Achene		Endosperm	
Source	df	MS	Pr > F	MS	Pr > F
Hour	3	0.00149	*	0.00028	NS
Error a (Block(hour))	8	0.00014		0.00029	
Prime	1	0.00241	*	0.00000	NS
Hour x Prime	3	0.00042	NS	0.00003	NS
Error b	8	0.00023		0.00015	

Everglades

		Achene		Endosperm	
Source	df	MS	Pr > F	MS	Pr > F
Hour	3	0.00657	**	0.00028	NS
Error a (Block(hour))	8	0.00053		0.00014	
Prime	1	0.00957	**	0.00065	*
Hour x Prime	3	0.00042	NS	0.00012	NS
Error b	8	0.0003		0.00008	

PI 251245

		Achene		Endosperm	
Source	df	MS	Pr > F	MS	Pr > F
Hour	3	0.00149	NS	0.00012	NS
Error a (Block(hour))	8	0.00011		0.00034	
Prime	1	0.00139	NS	0.00032	NS
Hour x Prime	3	0.00002	NS	0.00002	NS
Error b	8	0.00045		0.00006	

Table A-8. The imbibition time of seeds maturated at two different temperatures and primed seeds of lettuce genotypes, after which the seeds were fixed.

	Imbibition time (hour)			
Genotype	20°/10°C	30/20°C	Primed	Nonprimed
Dark Green Boston	15	10~11	9, 13	9, 13
Everglades	11~14	11~14	5, 6	5, 14
PI 251245	20	6~7	3, 4	3, 17
Valmaine	NM[z]	NM	5, 12	5, 12
Floricos 83	NM	NM	5, 6	5, 12

[z]NM: not measured

Table A-9. Germination and time to 50% of final seed germination at 36°C of lettuce seeds matured at two different temperatures.

	Germination (%)		GT_{50} (hour)[y]	
Genotype	20°/10°C	30°/20°C	20°/10°C	30°/20°C
Dark Green Boston	2	74 **[z]	24	17
Everglades	50	98**	18	18
PI 251245	33	97**	26	12

[z]NS, *, ** Nonsignificant or significant at P=0.05 or 0.01, respectively, by F-test.

[y] GT_{50}: Time to 50% of final seed germination.

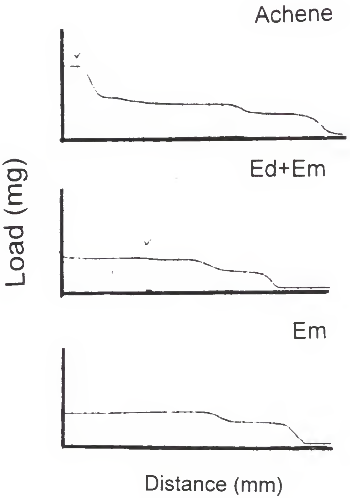


Figure A-1. A force-deformation curve of the three parts of lettuce seed recorded during the puncture test with the Instron Testing Machine.
Force of per square = 2.87mg.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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